HYPERSENSITIVE RESPONSE ELICITOR FRAGMENTS WHICH ARE ACTIVE BUT DO NOT ELICIT A HYPERSENSITIVE RESPONSE

This application claims benefit of U.S. Provisional Patent Application Serial No. 60/103,050, filed October 5, 1998.

FIELD OF THE INVENTION

The present invention relates to active fragments of a hypersensitive response elicitor which fragments do not elicit a hypersensitive response.

BACKGROUND OF THE INVENTION

Interactions between bacterial pathogens and their plant hosts generally fall into two categories: (1) compatible (pathogen-host), leading to intercellular bacterial growth, symptom development, and disease development in the host plant; and (2) incompatible (pathogen-nonhost), resulting in the hypersensitive response, a particular type of incompatible interaction occurring, without progressive disease symptoms. During compatible interactions on host plants, bacterial populations increase dramatically and progressive symptoms occur. During incompatible interactions, bacterial populations do not increase, and progressive symptoms do not occur.

The hypersensitive response is a rapid, localized necrosis that is associated with the active defense of plants against many pathogens (Kiraly, Z., "Defenses Triggered by the Invader: Hypersensitivity," pages 201-224 in: Plant Disease: An Advanced Treatise, Vol. 5, J.G. Horsfall and E.B. Cowling, ed. Academic Press New York (1980); Klement, Z., "Hypersensitivity," pages 149-177 in: Phytopathogenic Prokaryotes, Vol. 2, M.S. Mount and G.H. Lacy, ed. Academic Press, New York (1982)). The hypersensitive response elicited by bacteria is readily observed as a tissue collapse if high concentrations ($\geq 10^7$ cells/ml) of a limited host-range pathogen like *Pseudomonas syringae* or *Erwinia amylovora* are infiltrated into the leaves of nonhost plants (necrosis occurs only in isolated plant cells at lower levels of inoculum) (Klement, Z., "Rapid Detection of Pathogenicity of Phytopathogenic Pseudomonads," Nature 199:299-300; Klement, et al.,

"Hypersensitive Reaction Induced by Phytopathogenic Bacteria in the Tobacco Leaf," Phytopathology 54:474-477 (1963); Turner, et al., "The Quantitative Relation Between Plant and Bacterial Cells Involved in the Hypersensitive Reaction," Phytopathology 64:885-890 (1974); Klement, Z., "Hypersensitivity," pages 149-177 in Phytopathogenic Prokaryotes, Vol. 2., M.S. Mount and G.H. Lacy, ed. Academic Press, New York (1982)). The capacities to elicit the hypersensitive response in a nonhost and be pathogenic in a host appear linked. As noted by Klement, Z., "Hypersensitivity," pages 149-177 in Phytopathogenic Prokaryotes, Vol. 2., M.S. Mount and G.H. Lacy, ed. Academic Press, New York, these pathogens also cause physiologically similar, albeit delayed, necroses in their interactions with compatible hosts. Furthermore, the ability to produce the hypersensitive response or pathogenesis is dependent on a common set of genes, denoted *hrp* (Lindgren, P.B., et al., "Gene Cluster of *Pseudomonas syringae* pv. 'phaseolicola' Controls Pathogenicity of Bean Plants and Hypersensitivity on Nonhost Plants," J. Bacteriol. 168:512-22 (1986); Willis, D.K., et al., "*hrp* Genes of Phytopathogenic Bacteria," Mol. Plant-Microbe Interact. 4:132-138 (1991)). Consequently, the hypersensitive response may hold clues to both the nature of plant defense and the basis for bacterial pathogenicity.

The *hrp* genes are widespread in Gram-negative plant pathogens, where they are clustered, conserved, and in some cases interchangeable (Willis, D.K., et al., "*hrp* Genes of Phytopathogenic Bacteria," Mol. Plant-Microbe Interact. 4:132-138 (1991); Bonas, U., "*hrp* Genes of Phytopathogenic Bacteria," pages 79-98 in: Current Topics in Microbiology and Immunology: Bacterial Pathogenesis of Plants and Animals - Molecular and Cellular Mechanisms, J.L. Dangl, ed. Springer-Verlag, Berlin (1994)). Several *hrp* genes encode components of a protein secretion pathway similar to one used by *Yersinia, Shigella*, and *Salmonella* spp. to secrete proteins essential in animal diseases (Van Gijsegem, et al., "Evolutionary Conservation of Pathogenicity Determinants Among Plant and Animal Pathogenic Bacteria," Trends Microbiol. 1:175-180 (1993)). In *E. amylovora, P. syringae*, and *P. solanacearum*, *hrp* genes have been shown to control the production and secretion of glycine-rich, protein elicitors of the hypersensitive response (He, S.Y., et al. "Pseudomonas Syringae pv. Syringae HarpinPss: a Protein that is Secreted via the Hrp Pathway and Elicits the Hypersensitive Response in Plants," Cell 73:1255-1266 (1993), Wei, Z.-H.,

et al., "HrpI of *Erwinia amylovora* Functions in Secretion of Harpin and is a Member of a New Protein Family," J. Bacteriol. 175:7958-7967 (1993); Arlat, M. et al. "PopA1, a Protein Which Induces a Hypersensitive-like Response on Specific Petunia Genotypes, is Secreted via the Hrp Pathway of *Pseudomonas solanacearum,*" EMBO J. 13:543-553 (1994)).

The first of these proteins was discovered in *E. amylovora* Ea321, a bacterium that causes fire blight of rosaceous plants, and was designated harpin (Wei, Z.-M., et al, "Harpin, Elicitor of the Hypersensitive Response Produced by the Plant Pathogen *Erwinia amylovora*," Science 257:85-88 (1992)). Mutations in the encoding *hrpN* gene revealed that harpin is required for *E. amylovora* to elicit a hypersensitive response in nonhost tobacco leaves and incite disease symptoms in highly susceptible pear fruit. The *P. solanacearum* GMI1000 PopA1 protein has similar physical properties and also elicits the hypersensitive response in leaves of tobacco, which is not a host of that strain (Arlat, et al. "PopA1, a Protein Which Induces a Hypersensitive-like Response on Specific Petunia Genotypes, is Secreted via the Hrp Pathway of *Pseudomonas solanacearum,*" EMBO J. 13:543-53 (1994)). However, *P. solanacearum popA* mutants still elicit the hypersensitive response in tobacco and incite disease in tomato. Thus, the role of these glycine-rich hypersensitive response elicitors can vary widely among Gram-negative plant pathogens.

Other plant pathogenic hypersensitive response elicitors have been isolated, cloned, and sequenced. These include: *Erwinia chrysanthemi* (Bauer, et. al., "*Erwinia chrysanthemi* $Harpin_{Ech}$: Soft-Rot Pathogenesis," MPMI 8(4): 484-91 (1995)); *Erwinia carotovora* (Cui, et. al., "The RsmA$^-$ Mutants of *Erwinia carotovora* subsp. *carotovora* Strain Ecc71 Overexpress *hrp*N_{Ecc} and Elicit a Hypersensitive Reaction-like Response in Tobacco Leaves," MPMI 9(7): 565-73 (1996)); *Erwinia stewartii* (Ahmad, et. al., "Harpin is not Necessary for the Pathogenicity of *Erwinia stewartii* on Maize," 8th Int'l. Cong. Molec. Plant-Microb. Inter. July 14-19, 1996 and Ahmad, et. al., "Harpin is not Necessary for the Pathogenicity of *Erwinia stewartii* on Maize," Ann. Mtg. Am. Phytopath. Soc. July 27-31, 1996); and *Pseudomonas syringae* pv. *syringae* (WO 94/26782 to Cornell Research Foundation, Inc.).

The present invention seeks to identify fragments of hypersensitive response elicitor proteins or polypeptides, which fragments do not elicit a hypersensitive response but are active when utilized in conjunction with plants.

SUMMARY OF THE INVENTION

The present invention is directed to isolated fragments of an *Erwinia* hypersensitive response elicitor protein or polypeptide which fragments do not elicit a hypersensitive response in plants but are otherwise active when utilized in conjunction with plants. Also disclosed are isolated DNA molecules which encode such fragments.

The fragments of hypersensitive response elicitors according to the present invention have the following activity when utilized in conjunction with plants: imparting disease resistance to plants, enhancing plant growth and/or controlling insects. This involves applying the fragments in a non-infectious form to plants or plant seeds under conditions effective to impart disease resistance, to enhance plant growth, and/or to control insects on plants or plants grown from the plant seeds.

As an alternative to applying the fragments to plants or plant seeds in order to impart disease resistance, to enhance plant growth, and/or to control insects on plants, transgenic plants or plant seeds can be utilized. When utilizing transgenic plants, this involves providing a transgenic plant transformed with a DNA molecule encoding a fragment of a hypersensitive response elicitor protein or polypeptide in accordance with the present invention and growing the plant under conditions effective to impart disease resistance, to enhance plant growth, and/or to control insects in the plants or plants grown from the plant seeds. Alternatively, a transgenic plant seed transformed with the DNA molecule encoding such a fragment can be provided and planted in soil. A plant is then propagated under conditions effective to impart disease resistance, to enhance plant growth, and/or to control insects on plants or plants grown from the plant seeds.

BRIEF DESCRIPTION OF THE DRAWINGS

Figure 1 shows truncated proteins of the hypersensitive response elicitor protein or polypeptide.

Figure 2 shows a list of synthesized oligonucleotide primers for construction of truncated harpin proteins. N represents the N-terminus (5' region), and C represents the C-terminus (3' region). The primers correspond to the indicated sequence identification numbers for the present application: N1 (SEQ. ID. No. 1), N76 (SEQ. ID. No. 2), N99 (SEQ. ID. No. 3), N105 (SEQ. ID. No. 4), N110 (SEQ. ID. No. 5), N137 (SEQ. ID. No. 6), N150 (SEQ. ID. No. 7), N169 (SEQ. ID. No. 8), N210 (SEQ. ID. No. 9), N267 (SEQ. ID. No. 10), N343 (SEQ. ID. No. 11), C75 (SEQ. ID. No. 12), C104 (SEQ. ID. No. 13), C168 (SEQ. ID. No. 14), C180 (SEQ. ID. No. 15), C204 (SEQ. ID. No. 16), C209 (SEQ. ID. No. 17), C266 (SEQ. ID. No. 18), C342 (SEQ. ID. No. 19), and C403 (SEQ. ID. No. 20).

DETAILED DESCRIPTION OF THE INVENTION

The present invention is directed to isolated fragments of a hypersensitive response elicitor protein or polypeptide where the fragments do not elicit a hypersensitive response but have other activity in plants. Also disclosed are DNA molecules encoding such fragments as well as expression systems, host cells, and plants containing such molecules. Uses of the fragments themselves and the DNA molecules encoding them are disclosed.

The fragments of hypersensitive response elicitor polypeptides or proteins according to the present invention are derived from hypersensitive response elicitor polypeptides or proteins of a wide variety of fungal and bacterial pathogens. Such polypeptides or proteins are able to elicit local necrosis in plant tissue contacted by the elicitor. Examples of suitable bacterial sources of polypeptide or protein elicitors include *Erwinia*, *Pseudomonas*, and *Xanthomonas* species (e.g., the following bacteria: *Erwinia amylovora, Erwinia chrysanthemi, Erwinia stewartii, Erwinia carotovora, Pseudomonas syringae, Pseudomonas solancearum, Xanthomonas campestris*, and mixtures thereof).

An example of a fungal source of a hypersensitive response elicitor protein or polypeptide is *Phytophthora*. Suitable species of *Phytophthora* include *Phytophthora parasitica, Phytophthora cryptogea, Phytophthora cinnamomi, Phytophthora capsici, Phytophthora megasperma*, and *Phytophthora citrophthora*.

The hypersensitive response elicitor polypeptide or protein from *Erwinia chrysanthemi* has an amino acid sequence corresponding to SEQ. ID. No. 21 as follows:

```
Met Gln Ile Thr Ile Lys Ala His Ile Gly Gly Asp Leu Gly Val Ser
1               5                  10                  15

Gly Leu Gly Ala Gln Gly Leu Lys Gly Leu Asn Ser Ala Ala Ser Ser
            20                  25                  30

Leu Gly Ser Ser Val Asp Lys Leu Ser Ser Thr Ile Asp Lys Leu Thr
        35                  40                  45

Ser Ala Leu Thr Ser Met Met Phe Gly Gly Ala Leu Ala Gln Gly Leu
    50                  55                  60

Gly Ala Ser Ser Lys Gly Leu Gly Met Ser Asn Gln Leu Gly Gln Ser
65                  70                  75                  80

Phe Gly Asn Gly Ala Gln Gly Ala Ser Asn Leu Leu Ser Val Pro Lys
                85                  90                  95

Ser Gly Gly Asp Ala Leu Ser Lys Met Phe Asp Lys Ala Leu Asp Asp
            100                 105                 110

Leu Leu Gly His Asp Thr Val Thr Lys Leu Thr Asn Gln Ser Asn Gln
        115                 120                 125

Leu Ala Asn Ser Met Leu Asn Ala Ser Gln Met Thr Gln Gly Asn Met
    130                 135                 140

Asn Ala Phe Gly Ser Gly Val Asn Asn Ala Leu Ser Ser Ile Leu Gly
145                 150                 155                 160

Asn Gly Leu Gly Gln Ser Met Ser Gly Phe Ser Gln Pro Ser Leu Gly
                165                 170                 175

Ala Gly Gly Leu Gln Gly Leu Ser Gly Ala Gly Ala Phe Asn Gln Leu
            180                 185                 190

Gly Asn Ala Ile Gly Met Gly Val Gly Gln Asn Ala Ala Leu Ser Ala
        195                 200                 205

Leu Ser Asn Val Ser Thr His Val Asp Gly Asn Asn Arg His Phe Val
    210                 215                 220

Asp Lys Glu Asp Arg Gly Met Ala Lys Glu Ile Gly Gln Phe Met Asp
225                 230                 235                 240
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Gln Tyr Pro Glu Ile Phe Gly Lys Pro Glu Tyr Gln Lys Asp Gly Trp
                245                 250                 255

Ser Ser Pro Lys Thr Asp Asp Lys Ser Trp Ala Lys Ala Leu Ser Lys
            260                 265                 270

Pro Asp Asp Asp Gly Met Thr Gly Ala Ser Met Asp Lys Phe Arg Gln
        275                 280                 285

Ala Met Gly Met Ile Lys Ser Ala Val Ala Gly Asp Thr Gly Asn Thr
    290                 295                 300

Asn Leu Asn Leu Arg Gly Ala Gly Gly Ala Ser Leu Gly Ile Asp Ala
305                 310                 315                 320

Ala Val Val Gly Asp Lys Ile Ala Asn Met Ser Leu Gly Lys Leu Ala
                325                 330                 335

Asn Ala
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This hypersensitive response elicitor polypeptide or protein has a molecular weight of 34 kDa, is heat stable, has a glycine content of greater than 16%, and contains substantially no cysteine. The *Erwinia chrysanthemi* hypersensitive response elicitor polypeptide or protein is encoded by a DNA molecule having a nucleotide sequence corresponding to SEQ. ID. No. 22 as follows:

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CGATTTTACC CGGGTGAACG TGCTATGACC GACAGCATCA CGGTATTCGA CACCGTTACG      60
GCGTTTATGG CCGCGATGAA CCGGCATCAG GCGGCGCGCT GGTCGCCGCA ATCCGGCGTC     120
GATCTGGTAT TTCAGTTTGG GGACACCGGG CGTGAACTCA TGATGCAGAT TCAGCCGGGG     180
CAGCAATATC CCGGCATGTT GCGCACGCTG CTCGCTCGTC GTTATCAGCA GGCGGCAGAG     240
TGCGATGGCT GCCATCTGTG CCTGAACGGC AGCGATGTAT TGATCCTCTG GTGGCCGCTG     300
CCGTCGGATC CCGGCAGTTA TCCGCAGGTG ATCGAACGTT TGTTTGAACT GGCGGGAATG     360
ACGTTGCCGT CGCTATCCAT AGCACCGACG GCGCGTCCGC AGACAGGGAA CGGACGCGCC     420
CGATCATTAA GATAAAGGCG GCTTTTTTTA TTGCAAAACG GTAACGGTGA GGAACCGTTT     480
CACCGTCGGC GTCACTCAGT AACAAGTATC CATCATGATG CCTACATCGG GATCGGCGTG     540
GGCATCCGTT GCAGATACTT TTGCGAACAC CTGACATGAA TGAGGAAACG AAATTATGCA     600
AATTACGATC AAAGCGCACA TCGGCGGTGA TTTGGGCGTC TCCGGTCTGG GGCTGGGTGC     660
TCAGGGACTG AAAGGACTGA ATTCCGCGGC TTCATCGCTG GGTTCCAGCG TGGATAAACT     720
GAGCAGCACC ATCGATAAGT TGACCTCCGC GCTGACTTCG ATGATGTTTG GCGGCGCGCT     780
GGCGCAGGGG CTGGGCGCCA GCTCGAAGGG GCTGGGGATG AGCAATCAAC TGGGCCAGTC     840
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TTTCGGCAAT GGCGCGCAGG GTGCGAGCAA CCTGCTATCC GTACCGAAAT CCGGCGGCGA     900
TGCGTTGTCA AAAATGTTTG ATAAAGCGCT GGACGATCTG CTGGGTCATG ACACCGTGAC     960
CAAGCTGACT AACCAGAGCA ACCAACTGGC TAATTCAATG CTGAACGCCA GCCAGATGAC    1020
CCAGGGTAAT ATGAATGCGT TCGGCAGCGG TGTGAACAAC GCACTGTCGT CCATTCTCGG    1080
CAACGGTCTC GGCCAGTCGA TGAGTGGCTT CTCTCAGCCT TCTCTGGGGG CAGGCGGCTT    1140
GCAGGGCCTG AGCGGCGCGG GTGCATTCAA CCAGTTGGGT AATGCCATCG GCATGGGCGT    1200
GGGGCAGAAT GCTGCGCTGA GTGCGTTGAG TAACGTCAGC ACCCACGTAG ACGGTAACAA    1260
CCGCCACTTT GTAGATAAAG AAGATCGCGG CATGGCGAAA GAGATCGGCC AGTTTATGGA    1320
TCAGTATCCG GAAATATTCG GTAAACCGCA ATACCAGAAA GATGGCTGGA GTTCGCCGAA    1380
GACGGACGAC AAATCCTGGG CTAAAGCGCT GAGTAAACCG GATGATGACG GTATGACCGG    1440
CGCCAGCATG GACAAATTCC GTCAGGCGAT GGGTATGATC AAAAGCGCGG TGGCGGGTGA    1500
TACCGGCAAT ACCAACCTGA ACCTGCGTGG CGCGGGCGGT GCATCGCTGG GTATCGATGC    1560
GGCTGTCGTC GGCGATAAAA TAGCCAACAT GTCGCTGGGT AAGCTGGCCA ACGCCTGATA    1620
ATCTGTGCTG GCCTGATAAA GCGGAAACGA AAAAAGAGAC GGGGAAGCCT GTCTCTTTTC    1680
TTATTATGCG GTTTATGCGG TTACCTGGAC CGGTTAATCA TCGTCATCGA TCTGGTACAA    1740
ACGCACATTT TCCCGTTCAT TCGCGTCGTT ACGCGCCACA ATCGCGATGG CATCTTCCTC    1800
GTCGCTCAGA TTGCGCGGCT GATGGGGAAC GCCGGGTGGA ATATAGAGAA ACTCGCCGGC    1860
CAGATGGAGA CACGTCTGCG ATAAATCTGT GCCGTAACGT GTTTCTATCC GCCCCTTTAG    1920
CAGATAGATT GCGGTTTCGT AATCAACATG GTAATGCGGT TCCGCCTGTG CGCCGGCCGG    1980
GATCACCACA ATATTCATAG AAAGCTGTCT TGCACCTACC GTATCGCGGG AGATACCGAC    2040
AAAATAGGGC AGTTTTTGCG TGGTATCCGT GGGGTGTTCC GGCCTGACAA TCTTGAGTTG    2100
GTTCGTCATC ATCTTTCTCC ATCTGGGCGA CCTGATCGGT T                        2141
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The hypersensitive response elicitor polypeptide or protein derived from *Erwinia amylovora* has an amino acid sequence corresponding to SEQ. ID. No. 23 as follows:

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Met Ser Leu Asn Thr Ser Gly Leu Gly Ala Ser Thr Met Gln Ile Ser
1               5                  10                  15

Ile Gly Gly Ala Gly Gly Asn Asn Gly Leu Leu Gly Thr Ser Arg Gln
            20                  25                  30
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Asn Ala Gly Leu Gly Gly Asn Ser Ala Leu Gly Leu Gly Gly Gly Asn
          35                  40                  45

Gln Asn Asp Thr Val Asn Gln Leu Ala Gly Leu Leu Thr Gly Met Met
      50                  55                  60

Met Met Met Ser Met Met Gly Gly Gly Gly Leu Met Gly Gly Gly Leu
65                  70                  75                  80

Gly Gly Gly Leu Gly Asn Gly Leu Gly Gly Ser Gly Gly Leu Gly Glu
                85                  90                  95

Gly Leu Ser Asn Ala Leu Asn Asp Met Leu Gly Gly Ser Leu Asn Thr
            100                 105                 110

Leu Gly Ser Lys Gly Gly Asn Asn Thr Thr Ser Thr Thr Asn Ser Pro
          115                 120                 125

Leu Asp Gln Ala Leu Gly Ile Asn Ser Thr Ser Gln Asn Asp Asp Ser
      130                 135                 140

Thr Ser Gly Thr Asp Ser Thr Ser Asp Ser Ser Asp Pro Met Gln Gln
145                 150                 155                 160

Leu Leu Lys Met Phe Ser Glu Ile Met Gln Ser Leu Phe Gly Asp Gly
                165                 170                 175

Gln Asp Gly Thr Gln Gly Ser Ser Ser Gly Gly Lys Gln Pro Thr Glu
            180                 185                 190

Gly Glu Gln Asn Ala Tyr Lys Lys Gly Val Thr Asp Ala Leu Ser Gly
          195                 200                 205

Leu Met Gly Asn Gly Leu Ser Gln Leu Leu Gly Asn Gly Gly Leu Gly
      210                 215                 220

Gly Gly Gln Gly Gly Asn Ala Gly Thr Gly Leu Asp Gly Ser Ser Leu
225                 230                 235                 240

Gly Gly Lys Gly Leu Gln Asn Leu Ser Gly Pro Val Asp Tyr Gln Gln
                245                 250                 255

Leu Gly Asn Ala Val Gly Thr Gly Ile Gly Met Lys Ala Gly Ile Gln
            260                 265                 270

Ala Leu Asn Asp Ile Gly Thr His Arg His Ser Ser Thr Arg Ser Phe
          275                 280                 285

Val Asn Lys Gly Asp Arg Ala Met Ala Lys Glu Ile Gly Gln Phe Met
      290                 295                 300

Asp Gln Tyr Pro Glu Val Phe Gly Lys Pro Gln Tyr Gln Lys Gly Pro
305                 310                 315                 320

Gly Gln Glu Val Lys Thr Asp Asp Lys Ser Trp Ala Lys Ala Leu Ser
                325                 330                 335

Lys Pro Asp Asp Asp Gly Met Thr Pro Ala Ser Met Glu Gln Phe Asn
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                340                 345                 350

Lys Ala Lys Gly Met Ile Lys Arg Pro Met Ala Gly Asp Thr Gly Asn
        355                 360                 365

Gly Asn Leu Gln Ala Arg Gly Ala Gly Gly Ser Ser Leu Gly Ile Asp
    370                 375                 380

Ala Met Met Ala Gly Asp Ala Ile Asn Asn Met Ala Leu Gly Lys Leu
385                 390                 395                 400

Gly Ala Ala
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This hypersensitive response elicitor polypeptide or protein has a molecular weight of about 39 kDa, has a pI of approximately 4.3, and is heat stable at 100°C for at least 10 minutes. This hypersensitive response elicitor polypeptide or protein has substantially no cysteine. The hypersensitive response elicitor polypeptide or protein derived from *Erwinia amylovora* is more fully described in Wei, Z.-M., R. J. Laby, C. H. Zumoff, D. W. Bauer, S.-Y. He, A. Collmer, and S. V. Beer, "Harpin, Elicitor of the Hypersensitive Response Produced by the Plant Pathogen *Erwinia amylovora*," Science 257:85-88 (1992), which is hereby incorporated by reference. The DNA molecule encoding this polypeptide or protein has a nucleotide sequence corresponding to SEQ. ID. No. 24 as follows:

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AAGCTTCGGC ATGGCACGTT TGACCGTTGG GTCGGCAGGG TACGTTTGAA TTATTCATAA      60
GAGGAATACG TTATGAGTCT GAATACAAGT GGGCTGGGAG CGTCAACGAT GCAAATTTCT     120
ATCGGCGGTG CGGGCGGAAA TAACGGGTTG CTGGGTACCA GTCGCCAGAA TGCTGGGTTG     180
GGTGGCAATT CTGCACTGGG GCTGGGCGGC GGTAATCAAA ATGATACCGT CAATCAGCTG     240
GCTGGCTTAC TCACCGGCAT GATGATGATG ATGAGCATGA TGGGCGGTGG TGGGCTGATG     300
GGCGGTGGCT TAGGCGGTGG CTTAGGTAAT GGCTTGGGTG GCTCAGGTGG CCTGGGCGAA     360
GGACTGTCGA ACGCGCTGAA CGATATGTTA GGCGGTTCGC TGAACACGCT GGGCTCGAAA     420
GGCGGCAACA ATACCACTTC AACAACAAAT TCCCCGCTGG ACCAGGCGCT GGGTATTAAC     480
TCAACGTCCC AAAACGACGA TTCCACCTCC GGCACAGATT CCACCTCAGA CTCCAGCGAC     540
CCGATGCAGC AGCTGCTGAA GATGTTCAGC GAGATAATGC AAAGCCTGTT TGGTGATGGG     600
CAAGATGGCA CCCAGGGCAG TTCCTCTGGG GGCAAGCAGC CGACCGAAGG CGAGCAGAAC     660
GCCTATAAAA AAGGAGTCAC TGATGCGCTG TCGGGCCTGA TGGGTAATGG TCTGAGCCAG     720
CTCCTTGGCA ACGGGGGACT GGGAGGTGGT CAGGGCGGTA ATGCTGGCAC GGGTCTTGAC     780
GGTTCGTCGC TGGGCGGCAA AGGGCTGCAA AACCTGAGCG GGCCGGTGGA CTACCAGCAG     840
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TTAGGTAACG CCGTGGGTAC CGGTATCGGT ATGAAAGCGG GCATTCAGGC GCTGAATGAT     900
ATCGGTACGC ACAGGCACAG TTCAACCCGT TCTTTCGTCA ATAAAGGCGA TCGGGCGATG     960
GCGAAGGAAA TCGGTCAGTT CATGGACCAG TATCCTGAGG TGTTTGGCAA GCCGCAGTAC    1020
CAGAAAGGCC CGGGTCAGGA GGTGAAAACC GATGACAAAT CATGGGCAAA AGCACTGAGC    1080
AAGCCAGATG ACGACGGAAT GACACCAGCC AGTATGGAGC AGTTCAACAA AGCCAAGGGC    1140
ATGATCAAAA GGCCCATGGC GGGTGATACC GGCAACGGCA ACCTGCAGGC ACGCGGTGCC    1200
GGTGGTTCTT CGCTGGGTAT TGATGCCATG ATGGCCGGTG ATGCCATTAA CAATATGGCA    1260
CTTGGCAAGC TGGGCGCGGC TTAAGCTT                                       1288
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Another potentially suitable hypersensitive response elicitor from *Erwinia amylovora* is disclosed in U.S. Patent Application Serial No. 09/120,927, which is hereby incorporated by reference. The protein is encoded by a DNA molecule having a nucleic acid sequence of SEQ. ID. No. 25 as follows:

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ATGTCAATTC TTACGCTTAA CAACAATACC TCGTCCTCGC CGGGTCTGTT CCAGTCCGGG      60
GGGGACAACG GGCTTGGTGG TCATAATGCA AATTCTGCGT TGGGGCAACA ACCCATCGAT     120
CGGCAAACCA TTGAGCAAAT GGCTCAATTA TTGGCGGAAC TGTTAAAGTC ACTGCTATCG     180
CCACAATCAG GTAATGCGGC AACCGGAGCC GGTGGCAATG ACCAGACTAC AGGAGTTGGT     240
AACGCTGGCG GCCTGAACGG ACGAAAAGGC ACAGCAGGAA CCACTCCGCA GTCTGACAGT     300
CAGAACATGC TGAGTGAGAT GGGCAACAAC GGGCTGGATC AGGCCATCAC GCCCGATGGC     360
CAGGGCGGCG GGCAGATCGG CGATAATCCT TTACTGAAAG CCATGCTGAA GCTTATTGCA     420
CGCATGATGG ACGGCCAAAG CGATCAGTTT GGCCAACCTG GTACGGGCAA CAACAGTGCC     480
TCTTCCGGTA CTTCTTCATC TGGCGGTTCC CCTTTTAACG ATCTATCAGG GGGGAAGGCC     540
CCTTCCGGCA ACTCCCCTTC CGGCAACTAC TCTCCCGTCA GTACCTTCTC ACCCCCATCC     600
ACGCCAACGT CCCCTACCTC ACCGCTTGAT TTCCCTTCTT CTCCCACCAA AGCAGCCGGG     660
GGCAGCACGC CGGTAACCGA TCATCCTGAC CCTGTTGGTA GCGCGGGCAT CGGGGCCGGA     720
AATTCGGTGG CCTTCACCAG CGCCGGCGCT AATCAGACGG TGCTGCATGA CACCATTACC     780
GTGAAAGCGG GTCAGGTGTT TGATGGCAAA GGACAAACCT TCACCGCCGG TTCAGAATTA     840
GGCGATGGCG GCCAGTCTGA AAACCAGAAA CCGCTGTTTA TACTGGAAGA CGGTGCCAGC     900
CTGAAAAACG TCACCATGGG CGACGACGGG GCGGATGGTA TTCATCTTTA CGGTGATGCC     960
AAAATAGACA ATCTGCACGT CACCAACGTG GGTGAGGACG CGATTACCGT TAAGCCAAAC    1020
AGCGCGGGCA AAAAATCCCA CGTTGAAATC ACTAACAGTT CCTTCGAGCA CGCCTCTGAC    1080
AAGATCCTGC AGCTGAATGC CGATACTAAC CTGAGCGTTG ACAACGTGAA GGCCAAAGAC    1140
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TTTGGTACTT TTGTACGCAC TAACGGCGGT CAACAGGGTA ACTGGGATCT GAATCTGAGC    1200

CATATCAGCG CAGAAGACGG TAAGTTCTCG TTCGTTAAAA GCGATAGCGA GGGGCTAAAC    1260

GTCAATACCA GTGATATCTC ACTGGGTGAT GTTGAAAACC ACTACAAAGT GCCGATGTCC    1320

GCCAACCTGA AGGTGGCTGA ATGA                                           1344
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See GenBank Accession No. U94513. The isolated DNA molecule of the present invention encodes a hypersensitive response elicitor protein or polypeptide having an amino acid sequence of SEQ. ID. No. 26 as follows:

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Met Ser Ile Leu Thr Leu Asn Asn Asn Thr Ser Ser Ser Pro Gly Leu
1               5                  10                  15

Phe Gln Ser Gly Gly Asp Asn Gly Leu Gly Gly His Asn Ala Asn Ser
            20                  25                  30

Ala Leu Gly Gln Gln Pro Ile Asp Arg Gln Thr Ile Glu Gln Met Ala
        35                  40                  45

Gln Leu Leu Ala Glu Leu Leu Lys Ser Leu Leu Ser Pro Gln Ser Gly
    50                  55                  60

Asn Ala Ala Thr Gly Ala Gly Gly Asn Asp Gln Thr Thr Gly Val Gly
65                  70                  75                  80

Asn Ala Gly Gly Leu Asn Gly Arg Lys Gly Thr Ala Gly Thr Thr Pro
                85                  90                  95

Gln Ser Asp Ser Gln Asn Met Leu Ser Glu Met Gly Asn Asn Gly Leu
            100                 105                 110

Asp Gln Ala Ile Thr Pro Asp Gly Gln Gly Gly Gly Gln Ile Gly Asp
        115                 120                 125

Asn Pro Leu Leu Lys Ala Met Leu Lys Leu Ile Ala Arg Met Met Asp
    130                 135                 140

Gly Gln Ser Asp Gln Phe Gly Gln Pro Gly Thr Gly Asn Asn Ser Ala
145                 150                 155                 160

Ser Ser Gly Thr Ser Ser Ser Gly Gly Ser Pro Phe Asn Asp Leu Ser
                165                 170                 175

Gly Gly Lys Ala Pro Ser Gly Asn Ser Pro Ser Gly Asn Tyr Ser Pro
            180                 185                 190

Val Ser Thr Phe Ser Pro Pro Ser Thr Pro Thr Ser Pro Thr Ser Pro
        195                 200                 205

Leu Asp Phe Pro Ser Ser Pro Thr Lys Ala Ala Gly Gly Ser Thr Pro
    210                 215                 220
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Val Thr Asp His Pro Asp Pro Val Gly Ser Ala Gly Ile Gly Ala Gly
225                 230                 235                 240

Asn Ser Val Ala Phe Thr Ser Ala Gly Ala Asn Gln Thr Val Leu His
                245                 250                 255

Asp Thr Ile Thr Val Lys Ala Gly Gln Val Phe Asp Gly Lys Gly Gln
            260                 265                 270

Thr Phe Thr Ala Gly Ser Glu Leu Gly Asp Gly Gly Gln Ser Glu Asn
        275                 280                 285

Gln Lys Pro Leu Phe Ile Leu Glu Asp Gly Ala Ser Leu Lys Asn Val
    290                 295                 300

Thr Met Gly Asp Asp Gly Ala Asp Gly Ile His Leu Tyr Gly Asp Ala
305                 310                 315                 320

Lys Ile Asp Asn Leu His Val Thr Asn Val Gly Glu Asp Ala Ile Thr
                325                 330                 335

Val Lys Pro Asn Ser Ala Gly Lys Lys Ser His Val Glu Ile Thr Asn
            340                 345                 350

Ser Ser Phe Glu His Ala Ser Asp Lys Ile Leu Gln Leu Asn Ala Asp
        355                 360                 365

Thr Asn Leu Ser Val Asp Asn Val Lys Ala Lys Asp Phe Gly Thr Phe
    370                 375                 380

Val Arg Thr Asn Gly Gly Gln Gln Gly Asn Trp Asp Leu Asn Leu Ser
385                 390                 395                 400

His Ile Ser Ala Glu Asp Gly Lys Phe Ser Phe Val Lys Ser Asp Ser
                405                 410                 415

Glu Gly Leu Asn Val Asn Thr Ser Asp Ile Ser Leu Gly Asp Val Glu
            420                 425                 430

Asn His Tyr Lys Val Pro Met Ser Ala Asn Leu Lys Val Ala Glu
        435                 440                 445
```

This protein or polypeptide is acidic, rich in glycine and serine, and lacks cysteine. It is also heat stable, protease sensitive, and suppressed by inhibitors of plant metabolism. The protein or polypeptide of the present invention has a predicted molecular size of ca. 4.5 kDa.

Another potentially suitable hypersensitive response elicitor from *Erwinia amylovora* is disclosed in U.S. Patent Application Serial No. 09/120,663 which is hereby incorporated by reference. The protein is encoded by a DNA molecule having a nucleic acid sequence of SEQ. ID. No. 27 as follows:

ATGGAATTAA AATCACTGGG AACTGAACAC AAGGCGGCAG TACACACAGC GGCGCACAAC 60

CCTGTGGGGC ATGGTGTTGC CTTACAGCAG GGCAGCAGCA GCAGCAGCCC GCAAAATGCC 120

GCTGCATCAT TGGCGGCAGA AGGCAAAAAT CGTGGGAAAA TGCCGAGAAT TCACCAGCCA 180

TCTACTGCGG CTGATGGTAT CAGCGCTGCT CACCAGCAAA AGAAATCCTT CAGTCTCAGG 240

GGCTGTTTGG GGACGAAAAA ATTTTCCAGA TCGGCACCGC AGGGCCAGCC AGGTACCACC 300

CACAGCAAAG GGGCAACATT GCGCGATCTG CTGGCGCGGG ACGACGGCGA AACGCAGCAT 360

GAGGCGGCCG CGCCAGATGC GGCGCGTTTG ACCCGTTCGG GCGGCGTCAA ACGCCGCAAT 420

ATGGACGACA TGGCCGGGCG GCCAATGGTG AAAGGTGGCA GCGGCGAAGA TAAGGTACCA 480

ACGCAGCAAA AACGGCATCA GCTGAACAAT TTTGGCCAGA TGCGCCAAAC GATGTTGAGC 540

AAAATGGCTC ACCCGGCTTC AGCCAACGCC GGCGATCGCC TGCAGCATTC ACCGCCGCAC 600

ATCCCGGGTA GCCACCACGA AATCAAGGAA GAACCGGTTG GCTCCACCAG CAAGGCAACA 660

ACGGCCCACG CAGACAGAGT GGAAATCGCT CAGGAAGATG ACGACAGCGA ATTCCAGCAA 720

CTGCATCAAC AGCGGCTGGC GCGCGAACGG GAAAATCCAC CGCAGCCGCC CAAACTCGGC 780

GTTGCCACAC CGATTAGCGC CAGGTTTCAG CCCAAACTGA CTGCGGTTGC GGAAAGCGTC 840

CTTGAGGGGA CAGATACCAC GCAGTCACCC CTTAAGCCGC AATCAATGCT GAAAGGAAGT 900

GGAGCCGGGG TAACGCCGCT GGCGGTAACG CTGGATAAAG GCAAGTTGCA GCTGGCACCG 960

GATAATCCAC CCGCGCTCAA TACGTTGTTG AAGCAGACAT TGGGTAAAGA CACCCAGCAC 1020

TATCTGGCGC ACCATGCCAG CAGCGACGGT AGCCAGCATC TGCTGCTGGA CAACAAAGGC 1080

CACCTGTTTG ATATCAAAAG CACCGCCACC AGCTATAGCG TGCTGCACAA CAGCCACCCC 1140

GGTGAGATAA AGGGCAAGCT GGCGCAGGCG GGTACTGGCT CCGTCAGCGT AGACGGTAAA 1200

AGCGGCAAGA TCTCGCTGGG GAGCGGTACG CAAAGTCACA ACAAAACAAT GCTAAGCCAA 1260

CCGGGGGAAG CGCACCGTTC CTTATTAACC GGCATTTGGC AGCATCCTGC TGGCGCAGCG 1320

CGGCCGCAGG GCGAGTCAAT CCGCCTGCAT GACGACAAAA TTCATATCCT GCATCCGGAG 1380

CTGGGCGTAT GGCAATCTGC GGATAAAGAT ACCCACAGCC AGCTGTCTCG CCAGGCAGAC 1440

GGTAAGCTCT ATGCGCTGAA AGACAACCGT ACCCTGCAAA ACCTCTCCGA TAATAAATCC 1500

TCAGAAAAGC TGGTCGATAA AATCAAATCG TATTCCGTTG ATCAGCGGGG GCAGGTGGCG 1560

ATCCTGACGG ATACTCCCGG CCGCCATAAG ATGAGTATTA TGCCCTCGCT GGATGCTTCC 1620

CCGGAGAGCC ATATTTCCCT CAGCCTGCAT TTTGCCGATG CCCACCAGGG GTTATTGCAC 1680

GGGAAGTCGG AGCTTGAGGC ACAATCTGTC GCGATCAGCC ATGGGCGACT GGTTGTGGCC 1740

GATAGCGAAG GCAAGCTGTT TAGCGCCGCC ATTCCGAAGC AAGGGGATGG AAACGAACTG 1800

AAAATGAAAG CCATGCCTCA GCATGCGCTC GATGAACATT TTGGTCATGA CCACCAGATT 1860

TCTGGATTTT TCCATGACGA CCACGGCCAG CTTAATGCGC TGGTGAAAAA TAACTTCAGG 1920

CAGCAGCATG CCTGCCCGTT GGGTAACGAT CATCAGTTTC ACCCCGGCTG GAACCTGACT 1980

GATGCGCTGG TTATCGACAA TCAGCTGGGG CTGCATCATA CCAATCCTGA ACCGCATGAG 2040

ATTCTTGATA TGGGGCATTT AGGCAGCCTG GCGTTACAGG AGGGCAAGCT TCACTATTTT 2100

GACCAGCTGA CCAAAGGGTG GACTGGCGCG GAGTCAGATT GTAAGCAGCT GAAAAAAGGC 2160

CTGGATGGAG CAGCTTATCT ACTGAAAGAC GGTGAAGTGA AACGCCTGAA TATTAATCAG 2220

AGCACCTCCT CTATCAAGCA CGGAACGGAA AACGTTTTTT CGCTGCCGCA TGTGCGCAAT 2280

AAACCGGAGC CGGGAGATGC CCTGCAAGGG CTGAATAAAG ACGATAAGGC CCAGGCCATG 2340

GCGGTGATTG GGGTAAATAA ATACCTGGCG CTGACGGAAA AAGGGGACAT TCGCTCCTTC 2400

CAGATAAAAC CCGGCACCCA GCAGTTGGAG CGGCCGGCAC AAACTCTCAG CCGCGAAGGT 2460

ATCAGCGGCG AACTGAAAGA CATTCATGTC GACCACAAGC AGAACCTGTA TGCCTTGACC 2520

CACGAGGGAG AGGTGTTTCA TCAGCCGCGT GAAGCCTGGC AGAATGGTGC CGAAAGCAGC 2580

AGCTGGCACA AACTGGCGTT GCCACAGAGT GAAAGTAAGC TAAAAAGTCT GGACATGAGC 2640

CATGAGCACA AACCGATTGC CACCTTTGAA GACGGTAGCC AGCATCAGCT GAAGGCTGGC 2700

GGCTGGCACG CCTATGCGGC ACCTGAACGC GGGCCGCTGG CGGTGGGTAC CAGCGGTTCA 2760

CAAACCGTCT TTAACCGACT AATGCAGGGG GTGAAAGGCA AGGTGATCCC AGGCAGCGGG 2820

TTGACGGTTA AGCTCTCGGC TCAGACGGGG GGAATGACCG GCGCCGAAGG GCGCAAGGTC 2880

AGCAGTAAAT TTTCCGAAAG GATCCGCGCC TATGCGTTCA ACCCAACAAT GTCCACGCCG 2940

CGACCGATTA AAAATGCTGC TTATGCCACA CAGCACGGCT GGCAGGGGCG TGAGGGGTTG 3000

AAGCCGTTGT ACGAGATGCA GGGAGCGCTG ATTAAACAAC TGGATGCGCA TAACGTTCGT 3060

CATAACGCGC CACAGCCAGA TTTGCAGAGC AAACTGGAAA CTCTGGATTT AGGCGAACAT 3120

GGCGCAGAAT TGCTTAACGA CATGAAGCGC TTCCGCGACG AACTGGAGCA GAGTGCAACC 3180

CGTTCGGTGA CCGTTTTAGG TCAACATCAG GGAGTGCTAA AAAGCAACGG TGAAATCAAT 3240

AGCGAATTTA AGCCATCGCC CGGCAAGGCG TTGGTCCAGA GCTTTAACGT CAATCGCTCT 3300

GGTCAGGATC TAAGCAAGTC ACTGCAACAG GCAGTACATG CCACGCCGCC ATCCGCAGAG 3360

AGTAAACTGC AATCCATGCT GGGGCACTTT GTCAGTGCCG GGGTGGATAT GAGTCATCAG 3420

AAGGGCGAGA TCCCGCTGGG CCGCCAGCGC GATCCGAATG ATAAAACCGC ACTGACCAAA 3480

TCGCGTTTAA TTTTAGATAC CGTGACCATC GGTGAACTGC ATGAACTGGC CGATAAGGCG 3540

AAACTGGTAT CTGACCATAA ACCCGATGCC GATCAGATAA AACAGCTGCG CCAGCAGTTC 3600

GATACGCTGC GTGAAAAGCG GTATGAGAGC AATCCGGTGA AGCATTACAC CGATATGGGC 3660

TTCACCCATA ATAAGGCGCT GGAAGCAAAC TATGATGCGG TCAAAGCCTT TATCAATGCC 3720

TTTAAGAAAG AGCACCACGG CGTCAATCTG ACCACGCGTA CCGTACTGGA ATCACAGGGC 3780

AGTGCGGAGC TGGCGAAGAA GCTCAAGAAT ACGCTGTTGT CCCTGGACAG TGGTGAAAGT 3840

ATGAGCTTCA GCCGGTCATA TGGCGGGGGC GTCAGCACTG TCTTTGTGCC TACCCTTAGC 3900

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AAGAAGGTGC CAGTTCCGGT GATCCCCGGA GCCGGCATCA CGCTGGATCG CGCCTATAAC    3960
CTGAGCTTCA GTCGTACCAG CGGCGGATTG AACGTCAGTT TTGGCCGCGA CGGCGGGGTG    4020
AGTGGTAACA TCATGGTCGC TACCGGCCAT GATGTGATGC CCTATATGAC CGGTAAGAAA    4080
ACCAGTGCAG GTAACGCCAG TGACTGGTTG AGCGCAAAAC ATAAAATCAG CCCGGACTTG    4140
CGTATCGGCG CTGCTGTGAG TGGCACCCTG CAAGGAACGC TACAAAACAG CCTGAAGTTT    4200
AAGCTGACAG AGGATGAGCT GCCTGGCTTT ATCCATGGCT TGACGCATGG CACGTTGACC    4260
CCGGCAGAAC TGTTGCAAAA GGGGATCGAA CATCAGATGA AGCAGGGCAG CAAACTGACG    4320
TTTAGCGTCG ATACCTCGGC AAATCTGGAT CTGCGTGCCG GTATCAATCT GAACGAAGAC    4380
GGCAGTAAAC CAAATGGTGT CACTGCCCGT GTTTCTGCCG GGCTAAGTGC ATCGGCAAAC    4440
CTGGCCGCCG GCTCGCGTGA ACGCAGCACC ACCTCTGGCC AGTTTGGCAG CACGACTTCG    4500
GCCAGCAATA ACCGCCCAAC CTTCCTCAAC GGGGTCGGCG CGGGTGCTAA CCTGACGGCT    4560
GCTTTAGGGG TTGCCCATTC ATCTACGCAT GAAGGGAAAC CGGTCGGGAT CTTCCCGGCA    4620
TTTACCTCGA CCAATGTTTC GGCAGCGCTG GCGCTGGATA ACCGTACCTC ACAGAGTATC    4680
AGCCTGGAAT TGAAGCGCGC GGAGCCGGTG ACCAGCAACG ATATCAGCGA GTTGACCTCC    4740
ACGCTGGGAA AACACTTTAA GGATAGCGCC ACAACGAAGA TGCTTGCCGC TCTCAAAGAG    4800
TTAGATGACG CTAAGCCCGC TGAACAACTG CATATTTTAC AGCAGCATTT CAGTGCAAAA    4860
GATGTCGTCG GTGATGAACG CTACGAGGCG GTGCGCAACC TGAAAAAACT GGTGATACGT    4920
CAACAGGCTG CGGACAGCCA CAGCATGGAA TTAGGATCTG CCAGTCACAG CACGACCTAC    4980
AATAATCTGT CGAGAATAAA TAATGACGGC ATTGTCGAGC TGCTACACAA ACATTTCGAT    5040
GCGGCATTAC CAGCAAGCAG TGCCAAACGT CTTGGTGAAA TGATGAATAA CGATCCGGCA    5100
CTGAAAGATA TTATTAAGCA GCTGCAAAGT ACGCCGTTCA GCAGCGCCAG CGTGTCGATG    5160
GAGCTGAAAG ATGGTCTGCG TGAGCAGACG GAAAAAGCAA TACTGGACGG TAAGGTCGGT    5220
CGTGAAGAAG TGGGAGTACT TTTCCAGGAT CGTAACAACT TGCGTGTTAA ATCGGTCAGC    5280
GTCAGTCAGT CCGTCAGCAA AAGCGAAGGC TTCAATACCC CAGCGCTGTT ACTGGGGACG    5340
AGCAACAGCG CTGCTATGAG CATGGAGCGC AACATCGGAA CCATTAATTT TAAATACGGC    5400
CAGGATCAGA ACACCCCACG GCGATTTACC CTGGAGGGTG GAATAGCTCA GGCTAATCCG    5460
CAGGTCGCAT CTGCGCTTAC TGATTTGAAG AAGGAAGGGC TGGAAATGAA GAGCTAA       5517
```

This DNA molecule is known as the dspE gene for *Erwinia amylovora*. This isolated DNA molecule of the present invention encodes a protein or polypeptide which elicits a plant pathogen's hypersensitive response having an amino acid sequence of SEQ. ID. No. 28 as follows:

```
Met Glu Leu Lys Ser Leu Gly Thr Glu His Lys Ala Ala Val His Thr
1               5                  10                  15

Ala Ala His Asn Pro Val Gly His Gly Val Ala Leu Gln Gln Gly Ser
            20                  25                  30

Ser Ser Ser Ser Pro Gln Asn Ala Ala Ala Ser Leu Ala Ala Glu Gly
        35                  40                  45

Lys Asn Arg Gly Lys Met Pro Arg Ile His Gln Pro Ser Thr Ala Ala
    50                  55                  60

Asp Gly Ile Ser Ala Ala His Gln Gln Lys Lys Ser Phe Ser Leu Arg
65                  70                  75                  80

Gly Cys Leu Gly Thr Lys Lys Phe Ser Arg Ser Ala Pro Gln Gly Gln
                85                  90                  95

Pro Gly Thr Thr His Ser Lys Gly Ala Thr Leu Arg Asp Leu Leu Ala
            100                 105                 110

Arg Asp Asp Gly Glu Thr Gln His Glu Ala Ala Ala Pro Asp Ala Ala
        115                 120                 125

Arg Leu Thr Arg Ser Gly Gly Val Lys Arg Arg Asn Met Asp Asp Met
    130                 135                 140

Ala Gly Arg Pro Met Val Lys Gly Gly Ser Gly Glu Asp Lys Val Pro
145                 150                 155                 160

Thr Gln Gln Lys Arg His Gln Leu Asn Asn Phe Gly Gln Met Arg Gln
                165                 170                 175

Thr Met Leu Ser Lys Met Ala His Pro Ala Ser Ala Asn Ala Gly Asp
            180                 185                 190

Arg Leu Gln His Ser Pro Pro His Ile Pro Gly Ser His His Glu Ile
        195                 200                 205

Lys Glu Glu Pro Val Gly Ser Thr Ser Lys Ala Thr Thr Ala His Ala
    210                 215                 220

Asp Arg Val Glu Ile Ala Gln Glu Asp Asp Asp Ser Glu Phe Gln Gln
225                 230                 235                 240

Leu His Gln Gln Arg Leu Ala Arg Glu Arg Glu Asn Pro Pro Gln Pro
                245                 250                 255

Pro Lys Leu Gly Val Ala Thr Pro Ile Ser Ala Arg Phe Gln Pro Lys
            260                 265                 270

Leu Thr Ala Val Ala Glu Ser Val Leu Glu Gly Thr Asp Thr Thr Gln
        275                 280                 285

Ser Pro Leu Lys Pro Gln Ser Met Leu Lys Gly Ser Gly Ala Gly Val
    290                 295                 300

Thr Pro Leu Ala Val Thr Leu Asp Lys Gly Lys Leu Gln Leu Ala Pro
305                 310                 315                 320

Asp Asn Pro Pro Ala Leu Asn Thr Leu Leu Lys Gln Thr Leu Gly Lys
                325                 330                 335

Asp Thr Gln His Tyr Leu Ala His His Ala Ser Ser Asp Gly Ser Gln
            340                 345                 350
```

```
His Leu Leu Leu Asp Asn Lys Gly His Leu Phe Asp Ile Lys Ser Thr
        365                 360                 365

Ala Thr Ser Tyr Ser Val Leu His Asn Ser His Pro Gly Glu Ile Lys
    370                 375                 380

Gly Lys Leu Ala Gln Ala Gly Thr Gly Ser Val Ser Val Asp Gly Lys
385                 390                 395                 400

Ser Gly Lys Ile Ser Leu Gly Ser Gly Thr Gln Ser His Asn Lys Thr
                405                 410                 415

Met Leu Ser Gln Pro Gly Glu Ala His Arg Ser Leu Leu Thr Gly Ile
            420                 425                 430

Trp Gln His Pro Ala Gly Ala Ala Arg Pro Gln Gly Glu Ser Ile Arg
        435                 440                 445

Leu His Asp Asp Lys Ile His Ile Leu His Pro Glu Leu Gly Val Trp
    450                 455                 460

Gln Ser Ala Asp Lys Asp Thr His Ser Gln Leu Ser Arg Gln Ala Asp
465                 470                 475                 480

Gly Lys Leu Tyr Ala Leu Lys Asp Asn Arg Thr Leu Gln Asn Leu Ser
                485                 490                 495

Asp Asn Lys Ser Ser Glu Lys Leu Val Asp Lys Ile Lys Ser Tyr Ser
            500                 505                 510

Val Asp Gln Arg Gly Gln Val Ala Ile Leu Thr Asp Thr Pro Gly Arg
        515                 520                 525

His Lys Met Ser Ile Met Pro Ser Leu Asp Ala Ser Pro Glu Ser His
    530                 535                 540

Ile Ser Leu Ser Leu His Phe Ala Asp Ala His Gln Gly Leu Leu His
545                 550                 555                 560

Gly Lys Ser Glu Leu Glu Ala Gln Ser Val Ala Ile Ser His Gly Arg
                565                 570                 575

Leu Val Val Ala Asp Ser Glu Gly Lys Leu Phe Ser Ala Ala Ile Pro
            580                 585                 590

Lys Gln Gly Asp Gly Asn Glu Leu Lys Met Lys Ala Met Pro Gln His
        595                 600                 605

Ala Leu Asp Glu His Phe Gly His Asp His Gln Ile Ser Gly Phe Phe
    610                 615                 620

His Asp Asp His Gly Gln Leu Asn Ala Leu Val Lys Asn Asn Phe Arg
625                 630                 635                 640

Gln Gln His Ala Cys Pro Leu Gly Asn Asp His Gln Phe His Pro Gly
                645                 650                 655

Trp Asn Leu Thr Asp Ala Leu Val Ile Asp Asn Gln Leu Gly Leu His
            660                 665                 670

His Thr Asn Pro Glu Pro His Glu Ile Leu Asp Met Gly His Leu Gly
        675                 680                 685
```

Sub A5

```
Ser Leu Ala Leu Gln Glu Gly Lys Leu His Tyr Phe Asp Gln Leu Thr
    690                 695                 700

Lys Gly Trp Thr Gly Ala Glu Ser Asp Cys Lys Gln Leu Lys Lys Gly
705                 710                 715                 720

Leu Asp Gly Ala Ala Tyr Leu Leu Lys Asp Gly Glu Val Lys Arg Leu
                725                 730                 735

Asn Ile Asn Gln Ser Thr Ser Ser Ile Lys His Gly Thr Glu Asn Val
            740                 745                 750

Phe Ser Leu Pro His Val Arg Asn Lys Pro Glu Pro Gly Asp Ala Leu
        755                 760                 765

Gln Gly Leu Asn Lys Asp Asp Lys Ala Gln Ala Met Ala Val Ile Gly
    770                 775                 780

Val Asn Lys Tyr Leu Ala Leu Thr Glu Lys Gly Asp Ile Arg Ser Phe
785                 790                 795                 800

Gln Ile Lys Pro Gly Thr Gln Gln Leu Glu Arg Pro Ala Gln Thr Leu
                805                 810                 815

Ser Arg Glu Gly Ile Ser Gly Glu Leu Lys Asp Ile His Val Asp His
            820                 825                 830

Lys Gln Asn Leu Tyr Ala Leu Thr His Glu Gly Glu Val Phe His Gln
        835                 840                 845

Pro Arg Glu Ala Trp Gln Asn Gly Ala Glu Ser Ser Ser Trp His Lys
    850                 855                 860

Leu Ala Leu Pro Gln Ser Glu Ser Lys Leu Lys Ser Leu Asp Met Ser
865                 870                 875                 880

His Glu His Lys Pro Ile Ala Thr Phe Glu Asp Gly Ser Gln His Gln
                885                 890                 895

Leu Lys Ala Gly Gly Trp His Ala Tyr Ala Ala Pro Glu Arg Gly Pro
            900                 905                 910

Leu Ala Val Gly Thr Ser Gly Ser Gln Thr Val Phe Asn Arg Leu Met
        915                 920                 925

Gln Gly Val Lys Gly Lys Val Ile Pro Gly Ser Gly Leu Thr Val Lys
    930                 935                 940

Leu Ser Ala Gln Thr Gly Gly Met Thr Gly Ala Glu Gly Arg Lys Val
945                 950                 955                 960

Ser Ser Lys Phe Ser Glu Arg Ile Arg Ala Tyr Ala Phe Asn Pro Thr
                965                 970                 975

Met Ser Thr Pro Arg Pro Ile Lys Asn Ala Ala Tyr Ala Thr Gln His
            980                 985                 990

Gly Trp Gln Gly Arg Glu Gly Leu Lys Pro Leu Tyr Glu Met Gln Gly
        995                 1000                1005

Ala Leu Ile Lys Gln Leu Asp Ala His Asn Val Arg His Asn Ala Pro
    1010                1015                1020

Gln Pro Asp Leu Gln Ser Lys Leu Glu Thr Leu Asp Leu Gly Glu His
1025                1030                1035                1040
```

Gly Ala Glu Leu Leu Asn Asp Met Lys Arg Phe Arg Asp Glu Leu Glu
1045 1050 1055

Gln Ser Ala Thr Arg Ser Val Thr Val Leu Gly Gln His Gln Gly Val
1060 1065 1070

Leu Lys Ser Asn Gly Glu Ile Asn Ser Glu Phe Lys Pro Ser Pro Gly
1075 1080 1085

Lys Ala Leu Val Gln Ser Phe Asn Val Asn Arg Ser Gly Gln Asp Leu
1090 1095 1100

Ser Lys Ser Leu Gln Gln Ala Val His Ala Thr Pro Pro Ser Ala Glu
1105 1110 1115 1120

Ser Lys Leu Gln Ser Met Leu Gly His Phe Val Ser Ala Gly Val Asp
1125 1130 1135

Met Ser His Gln Lys Gly Glu Ile Pro Leu Gly Arg Gln Arg Asp Pro
1140 1145 1150

Asn Asp Lys Thr Ala Leu Thr Lys Ser Arg Leu Ile Leu Asp Thr Val
1155 1160 1165

Thr Ile Gly Glu Leu His Glu Leu Ala Asp Lys Ala Lys Leu Val Ser
1170 1175 1180

Asp His Lys Pro Asp Ala Asp Gln Ile Lys Gln Leu Arg Gln Gln Phe
1185 1190 1195 1200

Asp Thr Leu Arg Glu Lys Arg Tyr Glu Ser Asn Pro Val Lys His Tyr
1205 1210 1215

Thr Asp Met Gly Phe Thr His Asn Lys Ala Leu Glu Ala Asn Tyr Asp
1220 1225 1230

Ala Val Lys Ala Phe Ile Asn Ala Phe Lys Lys Glu His His Gly Val
1235 1240 1245

Asn Leu Thr Thr Arg Thr Val Leu Glu Ser Gln Gly Ser Ala Glu Leu
1250 1255 1260

Ala Lys Lys Leu Lys Asn Thr Leu Leu Ser Leu Asp Ser Gly Glu Ser
1265 1270 1275 1280

Met Ser Phe Ser Arg Ser Tyr Gly Gly Gly Val Ser Thr Val Phe Val
1285 1290 1295

Pro Thr Leu Ser Lys Lys Val Pro Val Pro Val Ile Pro Gly Ala Gly
1300 1305 1310

Ile Thr Leu Asp Arg Ala Tyr Asn Leu Ser Phe Ser Arg Thr Ser Gly
1315 1320 1325

Gly Leu Asn Val Ser Phe Gly Arg Asp Gly Gly Val Ser Gly Asn Ile
1330 1335 1340

Met Val Ala Thr Gly His Asp Val Met Pro Tyr Met Thr Gly Lys Lys
1345 1350 1355 1360

Thr Ser Ala Gly Asn Ala Ser Asp Trp Leu Ser Ala Lys His Lys Ile
1365 1370 1375

```
Ser Pro Asp Leu Arg Ile Gly Ala Ala Val Ser Gly Thr Leu Gln Gly
          1380                1385                1390

Thr Leu Gln Asn Ser Leu Lys Phe Lys Leu Thr Glu Asp Glu Leu Pro
      1395                1400                1405

Gly Phe Ile His Gly Leu Thr His Gly Thr Leu Thr Pro Ala Glu Leu
  1410                1415                1420

Leu Gln Lys Gly Ile Glu His Gln Met Lys Gln Gly Ser Lys Leu Thr
1425                1430                1435                1440

Phe Ser Val Asp Thr Ser Ala Asn Leu Asp Leu Arg Ala Gly Ile Asn
                1445                1450                1455

Leu Asn Glu Asp Gly Ser Lys Pro Asn Gly Val Thr Ala Arg Val Ser
          1460                1465                1470

Ala Gly Leu Ser Ala Ser Ala Asn Leu Ala Ala Gly Ser Arg Glu Arg
      1475                1480                1485

Ser Thr Thr Ser Gly Gln Phe Gly Ser Thr Thr Ser Ala Ser Asn Asn
  1490                1495                1500

Arg Pro Thr Phe Leu Asn Gly Val Gly Ala Gly Ala Asn Leu Thr Ala
1505                1510                1515                1520

Ala Leu Gly Val Ala His Ser Ser Thr His Glu Gly Lys Pro Val Gly
                1525                1530                1535

Ile Phe Pro Ala Phe Thr Ser Thr Asn Val Ser Ala Ala Leu Ala Leu
          1540                1545                1550

Asp Asn Arg Thr Ser Gln Ser Ile Ser Leu Glu Leu Lys Arg Ala Glu
      1555                1560                1565

Pro Val Thr Ser Asn Asp Ile Ser Glu Leu Thr Ser Thr Leu Gly Lys
  1570                1575                1580

His Phe Lys Asp Ser Ala Thr Thr Lys Met Leu Ala Ala Leu Lys Glu
1585                1590                1595                1600

Leu Asp Asp Ala Lys Pro Ala Glu Gln Leu His Ile Leu Gln Gln His
                1605                1610                1615

Phe Ser Ala Lys Asp Val Val Gly Asp Glu Arg Tyr Glu Ala Val Arg
          1620                1625                1630

Asn Leu Lys Lys Leu Val Ile Arg Gln Gln Ala Ala Asp Ser His Ser
      1635                1640                1645

Met Glu Leu Gly Ser Ala Ser His Ser Thr Thr Tyr Asn Asn Leu Ser
  1650                1655                1660

Arg Ile Asn Asn Asp Gly Ile Val Glu Leu Leu His Lys His Phe Asp
1665                1670                1675                1680

Ala Ala Leu Pro Ala Ser Ser Ala Lys Arg Leu Gly Glu Met Met Asn
                1685                1690                1695

Asn Asp Pro Ala Leu Lys Asp Ile Ile Lys Gln Leu Gln Ser Thr Pro
          1700                1705                1710

Phe Ser Ser Ala Ser Val Ser Met Glu Leu Lys Asp Gly Leu Arg Glu
      1715                1720                1725
```

```
Gln Thr Glu Lys Ala Ile Leu Asp Gly Lys Val Gly Arg Glu Glu Val
    1730                1735                1740

Gly Val Leu Phe Gln Asp Arg Asn Asn Leu Arg Val Lys Ser Val Ser
1745                1750                1755                1760

Val Ser Gln Ser Val Ser Lys Ser Glu Gly Phe Asn Thr Pro Ala Leu
                1765                1770                1775

Leu Leu Gly Thr Ser Asn Ser Ala Ala Met Ser Met Glu Arg Asn Ile
            1780                1785                1790

Gly Thr Ile Asn Phe Lys Tyr Gly Gln Asp Gln Asn Thr Pro Arg Arg
        1795                1800                1805

Phe Thr Leu Glu Gly Gly Ile Ala Gln Ala Asn Pro Gln Val Ala Ser
    1810                1815                1820

Ala Leu Thr Asp Leu Lys Lys Glu Gly Leu Glu Met Lys Ser
1825                1830                1835
```

This protein or polypeptide is about 198 kDa and has a pI of 8.98.

The present invention relates to an isolated DNA molecule having a nucleotide sequence of SEQ. ID. No. 29 as follows:

```
ATGACATCGT CACAGCAGCG GGTTGAAAGG TTTTTACAGT ATTTCTCCGC CGGGTGTAAA      60
ACGCCCATAC ATCTGAAAGA CGGGGTGTGC GCCCTGTATA ACGAACAAGA TGAGGAGGCG     120
GCGGTGCTGG AAGTACCGCA ACACAGCGAC AGCCTGTTAC TACACTGCCG AATCATTGAG     180
GCTGACCCAC AAACTTCAAT AACCCTGTAT TCGATGCTAT TACAGCTGAA TTTTGAAATG     240
GCGGCCATGC GCGGCTGTTG GCTGGCGCTG GATGAACTGC ACAACGTGCG TTTATGTTTT     300
CAGCAGTCGC TGGAGCATCT GGATGAAGCA AGTTTTAGCG ATATCGTTAG CGGCTTCATC     360
GAACATGCGG CAGAAGTGCG TGAGTATATA GCGCAATTAG ACGAGAGTAG CGCGGCATAA     420
```

This is known as the dspF gene. This isolated DNA molecule of the present invention encodes a hypersensitive response elicitor protein or polypeptide having an amino acid sequence of SEQ. ID. No. 30 as follows:

```
Met Thr Ser Ser Gln Gln Arg Val Glu Arg Phe Leu Gln Tyr Phe Ser
1               5                   10                  15

Ala Gly Cys Lys Thr Pro Ile His Leu Lys Asp Gly Val Cys Ala Leu
            20                  25                  30

Tyr Asn Glu Gln Asp Glu Glu Ala Ala Val Leu Glu Val Pro Gln His
        35                  40                  45

Ser Asp Ser Leu Leu Leu His Cys Arg Ile Ile Glu Ala Asp Pro Gln
    50                  55                  60
```

```
Thr Ser Ile Thr Leu Tyr Ser Met Leu Leu Gln Leu Asn Phe Glu Met
65                  70                  75                  80

Ala Ala Met Arg Gly Cys Trp Leu Ala Leu Asp Glu Leu His Asn Val
                85                  90                  95

Arg Leu Cys Phe Gln Gln Ser Leu Glu His Leu Asp Glu Ala Ser Phe
            100                 105                 110

Ser Asp Ile Val Ser Gly Phe Ile Glu His Ala Ala Glu Val Arg Glu
        115                 120                 125

Tyr Ile Ala Gln Leu Asp Glu Ser Ser Ala Ala
    130                 135
```

This protein or polypeptide is about 16 kDa and has a pI of 4.45.

The hypersensitive response elicitor polypeptide or protein derived from *Pseudomonas syringae* has an amino acid sequence corresponding to SEQ. ID. No. 31 as follows:

```
Met Gln Ser Leu Ser Leu Asn Ser Ser Ser Leu Gln Thr Pro Ala Met
1               5                   10                  15

Ala Leu Val Leu Val Arg Pro Glu Ala Glu Thr Thr Gly Ser Thr Ser
            20                  25                  30

Ser Lys Ala Leu Gln Glu Val Val Val Lys Leu Ala Glu Glu Leu Met
        35                  40                  45

Arg Asn Gly Gln Leu Asp Asp Ser Ser Pro Leu Gly Lys Leu Leu Ala
    50                  55                  60

Lys Ser Met Ala Ala Asp Gly Lys Ala Gly Gly Gly Ile Glu Asp Val
65                  70                  75                  80

Ile Ala Ala Leu Asp Lys Leu Ile His Glu Lys Leu Gly Asp Asn Phe
                85                  90                  95

Gly Ala Ser Ala Asp Ser Ala Ser Gly Thr Gly Gln Gln Asp Leu Met
            100                 105                 110

Thr Gln Val Leu Asn Gly Leu Ala Lys Ser Met Leu Asp Asp Leu Leu
        115                 120                 125

Thr Lys Gln Asp Gly Gly Thr Ser Phe Ser Glu Asp Asp Met Pro Met
    130                 135                 140

Leu Asn Lys Ile Ala Gln Phe Met Asp Asp Asn Pro Ala Gln Phe Pro
145                 150                 155                 160

Lys Pro Asp Ser Gly Ser Trp Val Asn Glu Leu Lys Glu Asp Asn Phe
                165                 170                 175

Leu Asp Gly Asp Glu Thr Ala Ala Phe Arg Ser Ala Leu Asp Ile Ile
            180                 185                 190
```

```
Gly Gln Gln Leu Gly Asn Gln Gln Ser Asp Ala Gly Ser Leu Ala Gly
        195                 200                 205

Thr Gly Gly Gly Leu Gly Thr Pro Ser Ser Phe Ser Asn Asn Ser Ser
    210                 215                 220

Val Met Gly Asp Pro Leu Ile Asp Ala Asn Thr Gly Pro Gly Asp Ser
225                 230                 235                 240

Gly Asn Thr Arg Gly Glu Ala Gly Gln Leu Ile Gly Glu Leu Ile Asp
                245                 250                 255

Arg Gly Leu Gln Ser Val Leu Ala Gly Gly Gly Leu Gly Thr Pro Val
            260                 265                 270

Asn Thr Pro Gln Thr Gly Thr Ser Ala Asn Gly Gly Gln Ser Ala Gln
        275                 280                 285

Asp Leu Asp Gln Leu Leu Gly Gly Leu Leu Leu Lys Gly Leu Glu Ala
    290                 295                 300

Thr Leu Lys Asp Ala Gly Gln Thr Gly Thr Asp Val Gln Ser Ser Ala
305                 310                 315                 320

Ala Gln Ile Ala Thr Leu Leu Val Ser Thr Leu Leu Gln Gly Thr Arg
                325                 330                 335

Asn Gln Ala Ala Ala
            340
```

This hypersensitive response elicitor polypeptide or protein has a molecular weight of 34-35 kDa. It is rich in glycine (about 13.5%) and lacks cysteine and tyrosine. Further information about the hypersensitive response elicitor derived from *Pseudomonas syringae* is found in He, S. Y., H. C. Huang, and A. Collmer, "*Pseudomonas syringae* pv. *syringae* $Harpin_{Pss}$: a Protein that is Secreted via the Hrp Pathway and Elicits the Hypersensitive Response in Plants," Cell 73:1255-1266 (1993), which is hereby incorporated by reference. The DNA molecule encoding the hypersensitive response elicitor from *Pseudomonas syringae* has a nucleotide sequence corresponding to SEQ. ID. No. 32 as follows:

```
ATGCAGAGTC TCAGTCTTAA CAGCAGCTCG CTGCAAACCC CGGCAATGGC CCTTGTCCTG      60

GTACGTCCTG AAGCCGAGAC GACTGGCAGT ACGTCGAGCA AGGCGCTTCA GGAAGTTGTC     120

GTGAAGCTGG CCGAGGAACT GATGCGCAAT GGTCAACTCG ACGACAGCTC GCCATTGGGA     180

AAACTGTTGG CCAAGTCGAT GGCCGCAGAT GGCAAGGCGG GCGGCGGTAT TGAGGATGTC     240

ATCGCTGCGC TGGACAAGCT GATCCATGAA AAGCTCGGTG ACAACTTCGG CGCGTCTGCG     300
```

GACAGCGCCT CGGGTACCGG ACAGCAGGAC CTGATGACTC AGGTGCTCAA TGGCCTGGCC 360

AAGTCGATGC TCGATGATCT TCTGACCAAG CAGGATGGCG GGACAAGCTT CTCCGAAGAC 420

GATATGCCGA TGCTGAACAA GATCGCGCAG TTCATGGATG ACAATCCCGC ACAGTTTCCC 480

AAGCCGGACT CGGGCTCCTG GGTGAACGAA CTCAAGGAAG ACAACTTCCT TGATGGCGAC 540

GAAACGGCTG CGTTCCGTTC GGCACTCGAC ATCATTGGCC AGCAACTGGG TAATCAGCAG 600

AGTGACGCTG GCAGTCTGGC AGGGACGGGT GGAGGTCTGG GCACTCCGAG CAGTTTTTCC 660

AACAACTCGT CCGTGATGGG TGATCCGCTG ATCGACGCCA ATACCGGTCC CGGTGACAGC 720

GGCAATACCC GTGGTGAAGC GGGGCAACTG ATCGGCGAGC TTATCGACCG TGGCCTGCAA 780

TCGGTATTGG CCGGTGGTGG ACTGGGCACA CCCGTAAACA CCCCGCAGAC CGGTACGTCG 840

GCGAATGGCG GACAGTCCGC TCAGGATCTT GATCAGTTGC TGGGCGGCTT GCTGCTCAAG 900

GGCCTGGAGG CAACGCTCAA GGATGCCGGG CAAACAGGCA CCGACGTGCA GTCGAGCGCT 960

GCGCAAATCG CCACCTTGCT GGTCAGTACG CTGCTGCAAG GCACCCGCAA TCAGGCTGCA 1020

GCCTGA 1026

Another potentially suitable hypersensitive response elicitor from *Pseudomonas syringae* is disclosed in U.S. Patent Application Serial No. 09/120,817, which is hereby incorporated by reference. The protein has a nucleotide sequence of SEQ. ID. No. 33 as follows:

TCCACTTCGC TGATTTTGAA ATTGGCAGAT TCATAGAAAC GTTCAGGTGT GGAAATCAGG 60

CTGAGTGCGC AGATTTCGTT GATAAGGGTG TGGTACTGGT CATTGTTGGT CATTTCAAGG 120

CCTCTGAGTG CGGTGCGGAG CAATACCAGT CTTCCTGCTG GCGTGTGCAC ACTGAGTCGC 180

AGGCATAGGC ATTTCAGTTC CTTGCGTTGG TTGGGCATAT AAAAAAAGGA ACTTTTAAAA 240

ACAGTGCAAT GAGATGCCGG CAAAACGGGA ACCGGTCGCT GCGCTTTGCC ACTCACTTCG 300

AGCAAGCTCA ACCCCAAACA TCCACATCCC TATCGAACGG ACAGCGATAC GGCCACTTGC 360

TCTGGTAAAC CCTGGAGCTG GCGTCGGTCC AATTGCCCAC TTAGCGAGGT AACGCAGCAT 420

GAGCATCGGC ATCACACCCC GGCCGCAACA GACCACCACG CCACTCGATT TTTCGGCGCT 480

AAGCGGCAAG AGTCCTCAAC CAAACACGTT CGGCGAGCAG AACACTCAGC AAGCGATCGA 540

CCCGAGTGCA CTGTTGTTCG GCAGCGACAC ACAGAAAGAC GTCAACTTCG GCACGCCCGA 600

CAGCACCGTC CAGAATCCGC AGGACGCCAG CAAGCCCAAC GACAGCCAGT CCAACATCGC 660

TAAATTGATC AGTGCATTGA TCATGTCGTT GCTGCAGATG CTCACCAACT CCAATAAAAA 720

GCAGGACACC AATCAGGAAC AGCCTGATAG CCAGGCTCCT TTCCAGAACA ACGGCGGGCT 780

```
CGGTACACCG TCGGCCGATA GCGGGGGCGG CGGTACACCG GATGCGACAG GTGGCGGCGG     840
CGGTGATACG CCAAGCGCAA CAGGCGGTGG CGGCGGTGAT ACTCCGACCG CAACAGGCGG     900
TGGCGGCAGC GGTGGCGGCG GCACACCCAC TGCAACAGGT GGCGGCAGCG GTGGCACACC     960
CACTGCAACA GGCGGTGGCG AGGGTGGCGT AACACCGCAA ATCACTCCGC AGTTGGCCAA    1020
CCCTAACCGT ACCTCAGGTA CTGGCTCGGT GTCGGACACC GCAGGTTCTA CCGAGCAAGC    1080
CGGCAAGATC AATGTGGTGA AAGACACCAT CAAGGTCGGC GCTGGCGAAG TCTTTGACGG    1140
CCACGGCGCA ACCTTCACTG CCGACAAATC TATGGGTAAC GGAGACCAGG GCGAAAATCA    1200
GAAGCCCATG TTCGAGCTGG CTGAAGGCGC TACGTTGAAG AATGTGAACC TGGGTGAGAA    1260
CGAGGTCGAT GGCATCCACG TGAAAGCCAA AAACGCTCAG GAAGTCACCA TTGACAACGT    1320
GCATGCCCAG AACGTCGGTG AAGACCTGAT TACGGTCAAA GGCGAGGGAG GCGCAGCGGT    1380
CACTAATCTG AACATCAAGA ACAGCAGTGC CAAAGGTGCA GACGACAAGG TTGTCCAGCT    1440
CAACGCCAAC ACTCACTTGA AAATCGACAA CTTCAAGGCC GACGATTTCG GCACGATGGT    1500
TCGCACCAAC GGTGGCAAGC AGTTTGATGA CATGAGCATC GAGCTGAACG GCATCGAAGC    1560
TAACCACGGC AAGTTCGCCC TGGTGAAAAG CGACAGTGAC GATCTGAAGC TGGCAACGGG    1620
CAACATCGCC ATGACCGACG TCAAACACGC CTACGATAAA ACCCAGGCAT CGACCCAACA    1680
CACCGAGCTT TGAATCCAGA CAAGTAGCTT GAAAAAAGGG GGTGGACTC                1729
```

This DNA molecule is known as the dspE gene for *Pseudomonas syringae*. This isolated DNA molecule of the present invention encodes a protein or polypeptide which elicits a plant pathogen's hypersensitive response having an amino acid sequence of SEQ. ID. No. 34 as follows:

```
Met Ser Ile Gly Ile Thr Pro Arg Pro Gln Gln Thr Thr Thr Pro Leu
1               5                  10                  15

Asp Phe Ser Ala Leu Ser Gly Lys Ser Pro Gln Pro Asn Thr Phe Gly
            20                  25                  30

Glu Gln Asn Thr Gln Gln Ala Ile Asp Pro Ser Ala Leu Leu Phe Gly
        35                  40                  45

Ser Asp Thr Gln Lys Asp Val Asn Phe Gly Thr Pro Asp Ser Thr Val
    50                  55                  60

Gln Asn Pro Gln Asp Ala Ser Lys Pro Asn Asp Ser Gln Ser Asn Ile
65                  70                  75                  80

Ala Lys Leu Ile Ser Ala Leu Ile Met Ser Leu Leu Gln Met Leu Thr
                85                  90                  95
```

Dub A8

```
Asn Ser Asn Lys Lys Gln Asp Thr Asn Gln Glu Gln Pro Asp Ser Gln
            100                 105                 110

Ala Pro Phe Gln Asn Asn Gly Gly Leu Gly Thr Pro Ser Ala Asp Ser
        115                 120                 125

Gly Gly Gly Gly Thr Pro Asp Ala Thr Gly Gly Gly Gly Gly Asp Thr
    130                 135                 140

Pro Ser Ala Thr Gly Gly Gly Gly Gly Asp Thr Pro Thr Ala Thr Gly
145                 150                 155                 160

Gly Gly Gly Ser Gly Gly Gly Gly Thr Pro Thr Ala Thr Gly Gly Gly
                165                 170                 175

Ser Gly Gly Thr Pro Thr Ala Thr Gly Gly Gly Glu Gly Gly Val Thr
            180                 185                 190

Pro Gln Ile Thr Pro Gln Leu Ala Asn Pro Asn Arg Thr Ser Gly Thr
        195                 200                 205

Gly Ser Val Ser Asp Thr Ala Gly Ser Thr Glu Gln Ala Gly Lys Ile
    210                 215                 220

Asn Val Val Lys Asp Thr Ile Lys Val Gly Ala Gly Glu Val Phe Asp
225                 230                 235                 240

Gly His Gly Ala Thr Phe Thr Ala Asp Lys Ser Met Gly Asn Gly Asp
                245                 250                 255

Gln Gly Glu Asn Gln Lys Pro Met Phe Glu Leu Ala Glu Gly Ala Thr
            260                 265                 270

Leu Lys Asn Val Asn Leu Gly Glu Asn Glu Val Asp Gly Ile His Val
        275                 280                 285

Lys Ala Lys Asn Ala Gln Glu Val Thr Ile Asp Asn Val His Ala Gln
    290                 295                 300

Asn Val Gly Glu Asp Leu Ile Thr Val Lys Gly Glu Gly Gly Ala Ala
305                 310                 315                 320

Val Thr Asn Leu Asn Ile Lys Asn Ser Ser Ala Lys Gly Ala Asp Asp
                325                 330                 335

Lys Val Val Gln Leu Asn Ala Asn Thr His Leu Lys Ile Asp Asn Phe
            340                 345                 350

Lys Ala Asp Asp Phe Gly Thr Met Val Arg Thr Asn Gly Gly Lys Gln
        355                 360                 365

Phe Asp Asp Met Ser Ile Glu Leu Asn Gly Ile Glu Ala Asn His Gly
    370                 375                 380

Lys Phe Ala Leu Val Lys Ser Asp Ser Asp Asp Leu Lys Leu Ala Thr
385                 390                 395                 400

Gly Asn Ile Ala Met Thr Asp Val Lys His Ala Tyr Asp Lys Thr Gln
                405                 410                 415
```

```
Ala Ser Thr Gln His Thr Glu Leu
420
```

This protein or polypeptide is about 42.9 kDa.

The hypersensitive response elicitor polypeptide or protein derived from *Pseudomonas solanacearum* has an amino acid sequence corresponding to SEQ. ID. No. 35 as follows:

```
Met Ser Val Gly Asn Ile Gln Ser Pro Ser Asn Leu Pro Gly Leu Gln
1               5                  10                  15

Asn Leu Asn Leu Asn Thr Asn Thr Asn Ser Gln Gln Ser Gly Gln Ser
            20                  25                  30

Val Gln Asp Leu Ile Lys Gln Val Glu Lys Asp Ile Leu Asn Ile Ile
        35                  40                  45

Ala Ala Leu Val Gln Lys Ala Ala Gln Ser Ala Gly Gly Asn Thr Gly
    50                  55                  60

Asn Thr Gly Asn Ala Pro Ala Lys Asp Gly Asn Ala Asn Ala Gly Ala
65                  70                  75                  80

Asn Asp Pro Ser Lys Asn Asp Pro Ser Lys Ser Gln Ala Pro Gln Ser
                85                  90                  95

Ala Asn Lys Thr Gly Asn Val Asp Asp Ala Asn Asn Gln Asp Pro Met
            100                 105                 110

Gln Ala Leu Met Gln Leu Leu Glu Asp Leu Val Lys Leu Leu Lys Ala
        115                 120                 125

Ala Leu His Met Gln Gln Pro Gly Gly Asn Asp Lys Gly Asn Gly Val
    130                 135                 140

Gly Gly Ala Asn Gly Ala Lys Gly Ala Gly Gly Gln Gly Gly Leu Ala
145                 150                 155                 160

Glu Ala Leu Gln Glu Ile Glu Gln Ile Leu Ala Gln Leu Gly Gly Gly
                165                 170                 175

Gly Ala Gly Ala Gly Gly Ala Gly Gly Gly Val Gly Gly Ala Gly Gly
            180                 185                 190

Ala Asp Gly Gly Ser Gly Ala Gly Gly Ala Gly Gly Ala Asn Gly Ala
        195                 200                 205

Asp Gly Gly Asn Gly Val Asn Gly Asn Gln Ala Asn Gly Pro Gln Asn
    210                 215                 220
```

```
Ala Gly Asp Val Asn Gly Ala Asn Gly Ala Asp Asp Gly Ser Glu Asp
225                 230                 235                 240

Gln Gly Gly Leu Thr Gly Val Leu Gln Lys Leu Met Lys Ile Leu Asn
                245                 250                 255

Ala Leu Val Gln Met Met Gln Gln Gly Gly Leu Gly Gly Gly Asn Gln
            260                 265                 270

Ala Gln Gly Gly Ser Lys Gly Ala Gly Asn Ala Ser Pro Ala Ser Gly
        275                 280                 285

Ala Asn Pro Gly Ala Asn Gln Pro Gly Ser Ala Asp Asp Gln Ser Ser
    290                 295                 300

Gly Gln Asn Asn Leu Gln Ser Gln Ile Met Asp Val Val Lys Glu Val
305                 310                 315                 320

Val Gln Ile Leu Gln Gln Met Leu Ala Ala Gln Asn Gly Gly Ser Gln
                325                 330                 335

Gln Ser Thr Ser Thr Gln Pro Met
            340
```

It is encoded by a DNA molecule having a nucleotide sequence corresponding SEQ. ID. No. 36 as follows:

```
ATGTCAGTCG GAAACATCCA GAGCCCGTCG AACCTCCCGG GTCTGCAGAA CCTGAACCTC      60
AACACCAACA CCAACAGCCA GCAATCGGGC CAGTCCGTGC AAGACCTGAT CAAGCAGGTC     120
GAGAAGGACA TCCTCAACAT CATCGCAGCC CTCGTGCAGA AGGCCGCACA GTCGGCGGGC     180
GGCAACACCG GTAACACCGG CAACGCGCCG GCGAAGGACG GCAATGCCAA CGCGGGCGCC     240
AACGACCCGA GCAAGAACGA CCCGAGCAAG AGCCAGGCTC CGCAGTCGGC CAACAAGACC     300
GGCAACGTCG ACGACGCCAA CAACCAGGAT CCGATGCAAG CGCTGATGCA GCTGCTGGAA     360
GACCTGGTGA AGCTGCTGAA GGCGGCCCTG CACATGCAGC AGCCCGGCGG CAATGACAAG     420
GGCAACGGCG TGGGCGGTGC CAACGGCGCC AAGGGTGCCG GCGGCCAGGG CGGCCTGGCC     480
GAAGCGCTGC AGGAGATCGA GCAGATCCTC GCCCAGCTCG GCGGCGGCGG TGCTGGCGCC     540
GGCGGCGCGG GTGGCGGTGT CGGCGGTGCT GGTGGCGCGG ATGGCGGCTC CGGTGCGGGT     600
GGCGCAGGCG GTGCGAACGG CGCCGACGGC GGCAATGGCG TGAACGGCAA CCAGGCGAAC     660
GGCCCGCAGA ACGCAGGCGA TGTCAACGGT GCCAACGGCG CGGATGACGG CAGCGAAGAC     720
CAGGGCGGCC TCACCGGCGT GCTGCAAAAG CTGATGAAGA TCCTGAACGC GCTGGTGCAG     780
ATGATGCAGC AAGGCGGCCT CGGCGGCGGC AACCAGGCGC AGGGCGGCTC GAAGGGTGCC     840
GGCAACGCCT CGCCGGCTTC CGGCGCGAAC CCGGGCGCGA ACCAGCCCGG TTCGGCGGAT     900
```

```
GATCAATCGT CCGGCCAGAA CAATCTGCAA TCCCAGATCA TGGATGTGGT GAAGGAGGTC     960

GTCCAGATCC TGCAGCAGAT GCTGGCGGCG CAGAACGGCG GCAGCCAGCA GTCCACCTCG    1020

ACGCAGCCGA TGTAA                                                     1035
```

Further information regarding the hypersensitive response elicitor polypeptide or protein derived from *Pseudomonas solanacearum* is set forth in Arlat, M., F. Van Gijsegem, J. C. Huet, J. C. Pemollet, and C. A. Boucher, "PopA1, a Protein which Induces a Hypersensitive-like Response in Specific Petunia Genotypes, is Secreted via the Hrp Pathway of *Pseudomonas solanacearum*," EMBO J. 13:543-533 (1994), which is hereby incorporated by reference.

The hypersensitive response elicitor polypeptide or protein from *Xanthomonas campestris* pv. glycines has an amino acid sequence corresponding to SEQ. ID. No. 37 as follows:

```
Thr Leu Ile Glu Leu Met Ile Val Val Ala Ile Ile Ala Ile Leu Ala
1               5                  10                  15

Ala Ile Ala Leu Pro Ala Tyr Gln Asp Tyr
            20                  25
```

This sequence is an amino terminal sequence having only 26 residues from the hypersensitive response elicitor polypeptide or protein of *Xanthomonas campestris* pv. glycines. It matches with fimbrial subunit proteins determined in other *Xanthomonas campestris* pathovars.

The hypersensitive response elicitor polypeptide or protein from *Xanthomonas campestris pv. pelargonii* is heat stable, protease sensitive, and has a molecular weight of 20 kDa. It includes an amino acid sequence corresponding to SEQ. ID. No. 38 as follows:

```
Ser Ser Gln Gln Ser Pro Ser Ala Gly Ser Glu Gln Gln Leu Asp Gln
1               5                  10                  15

Leu Leu Ala Met
            20
```

Isolation of *Erwinia carotovora* hypersensitive response elictor protein or polypeptide is described in Cui et al., "The RsmA Mutants of *Erwinia carotovora*

subsp. *carotovora* Strain Ecc71 Overexpress *hrp* N_{Ecc} and Elicit a Hypersensitive Reaction-like Response in Tobacco Leaves," MPMI, 9(7):565-73 (1996), which is hereby incorporated by reference. The hypersensitive response elicitor protein or polypeptide of *Erwinia stewartii* is set forth in Ahmad et al., "Harpin is Not Necessary for the Pathogenicity of *Erwinia stewartii* on Maize," 8th Int'l. Cong. Molec. Plant-Microbe Interact., July 14-19, 1996 and Ahmad, et al., "Harpin is Not Necessary for the Pathogenicity of *Erwinia stewartii* on Maize," Ann. Mtg. Am. Phytopath. Soc., July 27-31, 1996, which are hereby incorporated by reference.

Hypersensitive response elicitor proteins or polypeptides from *Phytophthora parasitica, Phytophthora cryptogea, Phytophthora cinnamoni, Phytophthora capsici, Phytophthora megasperma,* and *Phytophora citrophthora* are described in Kaman, et al., "Extracellular Protein Elicitors from Phytophthora: Most Specificity and Induction of Resistance to Bacterial and Fungal Phytopathogens," Molec. Plant-Microbe Interact., 6(1):15-25 (1993), Ricci et al., "Structure and Activity of Proteins from Pathogenic Fungi Phytophthora Eliciting Necrosis and Acquired Resistance in Tobacco," Eur. J. Biochem., 183:555-63 (1989), Ricci et al., "Differential Production of Parasiticein, and Elicitor of Necrosis and Resistance in Tobacco, by Isolates of Phytophthora parasitica," Plant Path. 41:298-307 (1992), Baillreul et al, "A New Elicitor of the Hypersensitive Response in Tobacco: A Fungal Glycoprotein Elicits Cell Death, Expression of Defence Genes, Production of Salicylic Acid, and Induction of Systemic Acquired Resistance," Plant J., 8(4):551-60 (1995), and Bonnet et al., "Acquired Resistance Triggered by Elicitors in Tobacco and Other Plants," Eur. J. Plant Path., 102:181-92 (1996), which are hereby incorporated by reference.

Another hypersensitive response elicitor in accordance with the present invention is from *Clavibacter michiganensis* subsp. *sepedonicus* which is fully described in U.S. Patent Application Serial No. 09/136,625, which is hereby incorporated by reference.

The above elicitors are exemplary. Other elicitors can be identified by growing fungi or bacteria that elicit a hypersensitive response under conditions which genes encoding an elicitor are expressed. Cell-free preparations from culture

supernatants can be tested for elicitor activity (i.e. local necrosis) by using them to infiltrate appropriate plant tissues.

Fragments of the above hypersensitive response elicitor polypeptides or proteins as well as fragments of full length elicitors from other pathogens are encompassed by the present invention.

Suitable fragments can be produced by several means. In the first, subclones of the gene encoding a known elicitor protein are produced by conventional molecular genetic manipulation by subcloning gene fragments. The subclones then are expressed *in vitro* or *in vivo* in bacterial cells to yield a smaller protein or peptide that can be tested for elicitor activity according to the procedure described below.

As an alternative, fragments of an elicitor protein can be produced by digestion of a full-length elicitor protein with proteolytic enzymes like chymotrypsin or *Staphylococcus* proteinase A, or trypsin. Different proteolytic enzymes are likely to cleave elicitor proteins at different sites based on the amino acid sequence of the elicitor protein. Some of the fragments that result from proteolysis may be active elicitors of resistance.

In another approach, based on knowledge of the primary structure of the protein, fragments of the elicitor protein gene may be synthesized by using the PCR technique together with specific sets of primers chosen to represent particular portions of the protein. These then would be cloned into an appropriate vector for expression of a truncated peptide or protein.

Chemical synthesis can also be used to make suitable fragments. Such a synthesis is carried out using known amino acid sequences for the elicitor being produced. Alternatively, subjecting a full length elicitor to high temperatures and pressures will produce fragments. These fragments can then be separated by conventional procedures (e.g., chromatography, SDS-PAGE).

An example of suitable fragments of a hypersensitive response elicitor which do not elicit a hypersensitive response include fragments of the *Erwinia amylovora* hypersensitive response elicitor. Suitable fragments include a C-terminal fragment of the amino acid sequence of SEQ. ID. No. 23, an N-terminal fragment of the amino acid sequence of SEQ. ID. No. 23, or an internal fragment of the amino acid sequence of SEQ. ID. No. 23. The C-terminal fragment of the amino acid

sequence of SEQ. ID. No. 23 can span the following amino acids of SEQ. ID. No. 23: 169 and 403, 210 and 403, 267 and 403, or 343 and 403. The internal fragment of the amino acid sequence of SEQ. ID. No. 23 can span the following amino acids of SEQ. ID. No. 23: 105 and 179, 137 and 166, 121 and 150, or 137 and 156. Other suitable fragments can be identified in accordance with the present invention.

Another example of a useful fragment of a hypersensitive response elicitor which fragment does not itself elicit a hypersensitive response is the protein fragment containing amino acids 190 to 294 of the amino acid sequence (SEQ. ID. No. 31) for the *Pseudomonas syringae* pv. *syringae* hypersensitive response elicitor. This fragment is useful in imparting disease resistance and enhancing plant growth.

Yet another example of a useful fragment of a hypersensitive response elicitor is the peptide having an amino acid sequence corresponding to SEQ. ID. No. 39. This peptide is derived from the hypersensitive response eliciting glycoprotein of *Phytophthora megasperma* and enhances plant growth.

Variants may be made by, for example, the deletion or addition of amino acids that have minimal influence on the properties, secondary structure, and hydropathic nature of the polypeptide. For example, a polypeptide may be conjugated to a signal (or leader) sequence at the N-terminal end of the protein which co-translationally or post-translationally directs transfer of the protein. The polypeptide may also be conjugated to a linker or other sequence for ease of synthesis, purification, or identification of the polypeptide.

The fragment of the present invention is preferably in isolated form (i.e. separated from its host organism) and more preferably produced in purified form (preferably at least about 60%, more preferably 80%, pure) by conventional techniques. Typically, the fragment of the present invention is produced but not secreted into the growth medium of recombinant host cells. Alternatively, the protein or polypeptide of the present invention is secreted into growth medium. In the case of unsecreted protein, to isolate the protein fragment, the host cell (e.g., *E. coli*) carrying a recombinant plasmid is propagated, lysed by sonication, heat, or chemical treatment, and the homogenate is centrifuged to remove bacterial debris. The supernatant is then subjected to heat treatment and the fragment is separated by centrifugation. The supernatant fraction containing the fragment is subjected to gel filtration in an

appropriately sized dextran or polyacrylamide column to separate the fragment. If necessary, the protein fraction may be further purified by ion exchange or HPLC.

The DNA molecule encoding the fragment of the hypersensitive response elicitor polypeptide or protein can be incorporated in cells using conventional recombinant DNA technology. Generally, this involves inserting the DNA molecule into an expression system to which the DNA molecule is heterologous (i.e. not normally present). The heterologous DNA molecule is inserted into the expression system or vector in proper sense orientation and correct reading frame. The vector contains the necessary elements for the transcription and translation of the inserted protein-coding sequences.

U.S. Patent No. 4,237,224 to Cohen and Boyer, which is hereby incorporated by reference, describes the production of expression systems in the form of recombinant plasmids using restriction enzyme cleavage and ligation with DNA ligase. These recombinant plasmids are then introduced by means of transformation and replicated in unicellular cultures including procaryotic organisms and eucaryotic cells grown in tissue culture.

Recombinant genes may also be introduced into viruses, such as vaccina virus. Recombinant viruses can be generated by transfection of plasmids into cells infected with virus.

Suitable vectors include, but are not limited to, the following viral vectors such as lambda vector system gt11, gt WES.tB, Charon 4, and plasmid vectors such as pBR322, pBR325, pACYC177, pACYC1084, pUC8, pUC9, pUC18, pUC19, pLG339, pR290, pKC37, pKC101, SV 40, pBluescript II SK +/- or KS +/- (see "Stratagene Cloning Systems" Catalog (1993) from Stratagene, La Jolla, Calif, which is hereby incorporated by reference), pQE, pIH821, pGEX, pET series (see F.W. Studier et. al., "Use of T7 RNA Polymerase to Direct Expression of Cloned Genes," Gene Expression Technology vol. 185 (1990), which is hereby incorporated by reference), and any derivatives thereof. Recombinant molecules can be introduced into cells via transformation, particularly transduction, conjugation, mobilization, or electroporation. The DNA sequences are cloned into the vector using standard cloning procedures in the art, as described by Sambrook et al., Molecular Cloning: A

Laboratory Manual, Cold Springs Laboratory, Cold Springs Harbor, New York (1989), which is hereby incorporated by reference.

A variety of host-vector systems may be utilized to express the protein-encoding sequence(s). Primarily, the vector system must be compatible with the host cell used. Host-vector systems include but are not limited to the following: bacteria transformed with bacteriophage DNA, plasmid DNA, or cosmid DNA; microorganisms such as yeast containing yeast vectors; mammalian cell systems infected with virus (e.g., vaccinia virus, adenovirus, etc.); insect cell systems infected with virus (e.g., baculovirus); and plant cells infected by bacteria. The expression elements of these vectors vary in their strength and specificities. Depending upon the host-vector system utilized, any one of a number of suitable transcription and translation elements can be used.

Different genetic signals and processing events control many levels of gene expression (e.g., DNA transcription and messenger RNA (mRNA) translation).

Transcription of DNA is dependent upon the presence of a promotor which is a DNA sequence that directs the binding of RNA polymerase and thereby promotes mRNA synthesis. The DNA sequences of eucaryotic promotors differ from those of procaryotic promotors. Furthermore, eucaryotic promotors and accompanying genetic signals may not be recognized in or may not function in a procaryotic system, and, further, procaryotic promotors are not recognized and do not function in eucaryotic cells.

Similarly, translation of mRNA in procaryotes depends upon the presence of the proper procaryotic signals which differ from those of eucaryotes. Efficient translation of mRNA in procaryotes requires a ribosome binding site called the Shine-Dalgarno ("SD") sequence on the mRNA. This sequence is a short nucleotide sequence of mRNA that is located before the start codon, usually AUG, which encodes the amino-terminal methionine of the protein. The SD sequences are complementary to the 3'-end of the 16S rRNA (ribosomal RNA) and probably promote binding of mRNA to ribosomes by duplexing with the rRNA to allow correct positioning of the ribosome. For a review on maximizing gene expression, see Roberts and Lauer, Methods in Enzymology, 68:473 (1979), which is hereby incorporated by reference.

Promotors vary in their "strength" (i.e. their ability to promote transcription). For the purposes of expressing a cloned gene, it is desirable to use strong promotors in order to obtain a high level of transcription and, hence, expression of the gene. Depending upon the host cell system utilized, any one of a number of suitable promotors may be used. For instance, when cloning in *E. coli*, its bacteriophages, or plasmids, promotors such as the T7 phage promotor, *lac* promotor, *trp* promotor, *rec*A promotor, ribosomal RNA promotor, the P_R and P_L promotors of coliphage lambda and others, including but not limited, to *lac*UV5, *omp*F, *bla*, *lpp*, and the like, may be used to direct high levels of transcription of adjacent DNA segments. Additionally, a hybrid *trp-lac*UV5 (*tac*) promotor or other *E. coli* promotors produced by recombinant DNA or other synthetic DNA techniques may be used to provide for transcription of the inserted gene.

Bacterial host cell strains and expression vectors may be chosen which inhibit the action of the promotor unless specifically induced. In certain operations, the addition of specific inducers is necessary for efficient transcription of the inserted DNA. For example, the *lac* operon is induced by the addition of lactose or IPTG (isopropylthio-beta-D-galactoside). A variety of other operons, such as *trp*, *pro*, etc., are under different controls.

Specific initiation signals are also required for efficient gene transcription and translation in procaryotic cells. These transcription and translation initiation signals may vary in "strength" as measured by the quantity of gene specific messenger RNA and protein synthesized, respectively. The DNA expression vector, which contains a promotor, may also contain any combination of various "strong" transcription and/or translation initiation signals. For instance, efficient translation in *E. coli* requires an SD sequence about 7-9 bases 5' to the initiation codon ("ATG") to provide a ribosome binding site. Thus, any SD-ATG combination that can be utilized by host cell ribosomes may be employed. Such combinations include but are not limited to the SD-ATG combination from the *cro* gene or the *N* gene of coliphage lambda, or from the *E. coli* tryptophan E, D, C, B or A genes. Additionally, any SD-ATG combination produced by recombinant DNA or other techniques involving incorporation of synthetic nucleotides may be used.

Once the isolated DNA molecule encoding the fragment of a hypersensitive response elicitor polypeptide or protein has been cloned into an expression system, it is ready to be incorporated into a host cell. Such incorporation can be carried out by the various forms of transformation noted above, depending upon the vector/host cell system. Suitable host cells include, but are not limited to, bacteria, virus, yeast, mammalian cells, insect, plant, and the like.

The present invention further relates to methods of imparting disease resistance to plants, enhancing plant growth, and/or effecting insect control for plants. These methods involve applying the fragment of a hypersensitive response elicitor polypeptide or protein which does not elicit a hypersensitive response in a non-infectious form to all or part of a plant or a plant seed under conditions effective for the fragment to impart disease resistance, enhance growth, and/or control insects. Alternatively, these fragments of a hypersensitive response elicitor protein or polypeptide can be applied to plants such that seeds recovered from such plants themselves are able to impart disease resistance in plants, to enhance plant growth, and/or to effect insect control.

As an alternative to applying a fragment of a hypersensitive response elicitor polypeptide or protein to plants or plant seeds in order to impart disease resistance in plants, to effect plant growth, and/or to control insects on the plants or plants grown from the seeds, transgenic plants or plant seeds can be utilized. When utilizing transgenic plants, this involves providing a transgenic plant transformed with a DNA molecule encoding a fragment of a hypersensitive response elicitor polypeptide or protein, which fragment does not elicit a hypersensitive response, and growing the plant under conditions effective to permit that DNA molecule to impart disease resistance to plants, to enhance plant growth, and/or to control insects. Alternatively, a transgenic plant seed transformed with a DNA molecule encoding a fragment of a hypersensitive response elicitor polypeptide or protein which fragment does not elicit a hypersensitive response can be provided and planted in soil. A plant is then propagated from the planted seed under conditions effective to permit that DNA molecule to impart disease resistance to plants, to enhance plant growth, and/or to control insects.

The embodiment of the present invention where the hypersensitive response elicitor polypeptide or protein is applied to the plant or plant seed can be carried out in a number of ways, including: 1) application of an isolated fragment or 2) application of bacteria which do not cause disease and are transformed with a gene encoding the fragment. In the latter embodiment, the fragment can be applied to plants or plant seeds by applying bacteria containing the DNA molecule encoding the fragment of the hypersensitive response elicitor polypeptide or protein which fragment does not elicit a hypersensitive response. Such bacteria must be capable of secreting or exporting the fragment so that the fragment can contact plant or plant seed cells. In these embodiments, the fragment is produced by the bacteria *in planta* or on seeds or just prior to introduction of the bacteria to the plants or plant seeds.

The methods of the present invention can be utilized to treat a wide variety of plants or their seeds to impart disease resistance, enhance growth, and/or control insects. Suitable plants include dicots and monocots. More particularly, useful crop plants can include: alfalfa, rice, wheat, barley, rye, cotton, sunflower, peanut, corn, potato, sweet potato, bean, pea, chicory, lettuce, endive, cabbage, brussel sprout, beet, parsnip, turnip, cauliflower, broccoli, radish, spinach, onion, garlic, eggplant, pepper, celery, carrot, squash, pumpkin, zucchini, cucumber, apple, pear, melon, citrus, strawberry, grape, raspberry, pineapple, soybean, tobacco, tomato, sorghum, and sugarcane. Examples of suitable ornamental plants are: *Arabidopsis thaliana*, *Saintpaulia*, petunia, pelargonium, poinsettia, chrysanthemum, carnation, and zinnia.

With regard to the use of the fragments of the hypersensitive response elicitor protein or polypeptide of the present invention in imparting disease resistance, absolute immunity against infection may not be conferred, but the severity of the disease is reduced and symptom development is delayed. Lesion number, lesion size, and extent of sporulation of fungal pathogens are all decreased. This method of imparting disease resistance has the potential for treating previously untreatable diseases, treating diseases systemically which might not be treated separately due to cost, and avoiding the use of infectious agents or environmentally harmful materials.

The method of imparting pathogen resistance to plants in accordance with the present invention is useful in imparting resistance to a wide variety of

pathogens including viruses, bacteria, and fungi. Resistance, *inter alia*, to the following viruses can be achieved by the method of the present invention: *Tobacco mosaic virus* and *Tomato mosaic virus*. Resistance, *inter alia*, to the following bacteria can also be imparted to plants in accordance with present invention: *Pseudomonas solanacearum*, *Pseudomonas syringae* pv. *tabaci*, and *Xanthamonas campestris* pv. *pelargonii*. Plants can be made resistant, *inter alia*, to the following fungi by use of the method of the present invention: *Fusarium oxysporum* and *Phytophthora infestans*.

With regard to the use of the fragments of the hypersensitive response elicitor protein or polypeptide of the present invention to enhance plant growth, various forms of plant growth enhancement or promotion can be achieved. This can occur as early as when plant growth begins from seeds or later in the life of a plant. For example, plant growth according to the present invention encompasses greater yield, increased quantity of seeds produced, increased percentage of seeds germinated, increased plant size, greater biomass, more and bigger fruit, earlier fruit coloration, and earlier fruit and plant maturation. As a result, the present invention provides significant economic benefit to growers. For example, early germination and early maturation permit crops to be grown in areas where short growing seasons would otherwise preclude their growth in that locale. Increased percentage of seed germination results in improved crop stands and more efficient seed use. Greater yield, increased size, and enhanced biomass production allow greater revenue generation from a given plot of land.

Another aspect of the present invention is directed to effecting any form of insect control for plants. For example, insect control according to the present invention encompasses preventing insects from contacting plants to which the hypersensitive response elicitor has been applied, preventing direct insect damage to plants by feeding injury, causing insects to depart from such plants, killing insects proximate to such plants, interfering with insect larval feeding on such plants, preventing insects from colonizing host plants, preventing colonizing insects from releasing phytotoxins, etc. The present invention also prevents subsequent disease damage to plants resulting from insect infection.

The present invention is effective against a wide variety of insects. European corn borer is a major pest of corn (dent and sweet corn) but also feeds on over 200 plant species including green, wax, and lima beans and edible soybeans, peppers, potato, and tomato plus many weed species. Additional insect larval feeding pests which damage a wide variety of vegetable crops include the following: beet armyworm, cabbage looper, corn ear worm, fall armyworm, diamondback moth, cabbage root maggot, onion maggot, seed corn maggot, pickleworm (melonworm), pepper maggot, tomato pinworm, and maggots. Collectively, this group of insect pests represents the most economically important group of pests for vegetable production worldwide.

The method of the present invention involving application of the fragment of a hypersensitive response elicitor polypeptide or protein, which fragment does not elicit a hypersensitive response, can be carried out through a variety of procedures when all or part of the plant is treated, including leaves, stems, roots, propagules (e.g., cuttings), etc. This may (but need not) involve infiltration of the fragment of the hypersensitive response elicitor polypeptide or protein into the plant. Suitable application methods include high or low pressure spraying, injection, and leaf abrasion proximate to when elicitor application takes place. When treating plant seeds or propagules (e.g., cuttings), in accordance with the application embodiment of the present invention, the fragment of the hypersensitive response elicitor protein or polypeptide, in accordance with present invention, can be applied by low or high pressure spraying, coating, immersion, or injection. Other suitable application procedures can be envisioned by those skilled in the art provided they are able to effect contact of the fragment with cells of the plant or plant seed. Once treated with the fragment of the hypersensitive response elicitor of the present invention, the seeds can be planted in natural or artificial soil and cultivated using conventional procedures to produce plants. After plants have been propagated from seeds treated in accordance with the present invention, the plants may be treated with one or more applications of the fragment of the hypersensitive response elicitor protein or polypeptide or whole elicitors to impart disease resistance to plants, to enhance plant growth, and/or to control insects on the plants.

The fragment of the hypersensitive response elicitor polypeptide or protein, in accordance with the present invention, can be applied to plants or plant seeds alone or in a mixture with other materials. Alternatively, the fragment can be applied separately to plants with other materials being applied at different times.

A composition suitable for treating plants or plant seeds in accordance with the application embodiment of the present invention contains a fragment of a hypersensitive response elicitor polypeptide or protein which fragment does not elicit a hypersensitive response in a carrier. Suitable carriers include water, aqueous solutions, slurries, or dry powders. In this embodiment, the composition contains greater than 500 nM of the fragment.

Although not required, this composition may contain additional additives including fertilizer, insecticide, fungicide, nematacide, and mixtures thereof. Suitable fertilizers include $(NH_4)_2NO_3$. An example of a suitable insecticide is Malathion. Useful fungicides include Captan.

Other suitable additives include buffering agents, wetting agents, coating agents, and abrading agents. These materials can be used to facilitate the process of the present invention. In addition, the hypersensitive response eliciting fragment can be applied to plant seeds with other conventional seed formulation and treatment materials, including clays and polysaccharides.

In the alternative embodiment of the present invention involving the use of transgenic plants and transgenic seeds, a fragment of a hypersensitive response elicitor need not be applied topically to the plants or seeds. Instead, transgenic plants transformed with a DNA molecule encoding such a fragment are produced according to procedures well known in the art.

The vector described above can be microinjected directly into plant cells by use of micropipettes to transfer mechanically the recombinant DNA. Crossway, Mol. Gen. Genetics, 202:179-85 (1985), which is hereby incorporated by reference. The genetic material may also be transferred into the plant cell using polyethylene glycol. Krens, et al., Nature, 296:72-74 (1982), which is hereby incorporated by reference.

Another approach to transforming plant cells with a gene which imparts resistance to pathogens is particle bombardment (also known as biolistic

transformation) of the host cell. This can be accomplished in one of several ways. The first involves propelling inert or biologically active particles at cells. This technique is disclosed in U.S. Patent Nos. 4,945,050, 5,036,006, and 5,100,792, all to Sanford et al., which are hereby incorporated by reference. Generally, this procedure involves propelling inert or biologically active particles at the cells under conditions effective to penetrate the outer surface of the cell and to be incorporated within the interior thereof. When inert particles are utilized, the vector can be introduced into the cell by coating the particles with the vector containing the heterologous DNA. Alternatively, the target cell can be surrounded by the vector so that the vector is carried into the cell by the wake of the particle. Biologically active particles (e.g., dried bacterial cells containing the vector and heterologous DNA) can also be propelled into plant cells.

Yet another method of introduction is fusion of protoplasts with other entities, either minicells, cells, lysosomes, or other fusible lipid-surfaced bodies. Fraley, et al., Proc. Natl. Acad. Sci. USA, 79:1859-63 (1982), which is hereby incorporated by reference.

The DNA molecule may also be introduced into the plant cells by electroporation. Fromm et al., Proc. Natl. Acad. Sci. USA, 82:5824 (1985), which is hereby incorporated by reference. In this technique, plant protoplasts are electroporated in the presence of plasmids containing the expression cassette. Electrical impulses of high field strength reversibly permeabilize biomembranes allowing the introduction of the plasmids. Electroporated plant protoplasts reform the cell wall, divide, and regenerate.

Another method of introducing the DNA molecule into plant cells is to infect a plant cell with *Agrobacterium tumefaciens* or *A. rhizogenes* previously transformed with the gene. Under appropriate conditions known in the art, the transformed plant cells are grown to form shoots or roots, and develop further into plants. Generally, this procedure involves inoculating the plant tissue with a suspension of bacteria and incubating the tissue for 48 to 72 hours on regeneration medium without antibiotics at 25-28°C.

Agrobacterium is a representative genus of the Gram-negative family Rhizobiaceae. Its species are responsible for crown gall (*A. tumefaciens*) and hairy

root disease (*A. rhizogenes*). The plant cells in crown gall tumors and hairy roots are induced to produce amino acid derivatives known as opines, which are catabolized only by the bacteria. The bacterial genes responsible for expression of opines are a convenient source of control elements for chimeric expression cassettes. In addition, assaying for the presence of opines can be used to identify transformed tissue.

Heterologous genetic sequences can be introduced into appropriate plant cells, by means of the Ti plasmid of *A. tumefaciens* or the Ri plasmid of *A. rhizogenes*. The Ti or Ri plasmid is transmitted to plant cells on infection by Agrobacterium and is stably integrated into the plant genome. J. Schell, Science, 237:1176-83 (1987), which is hereby incorporated by reference.

After transformation, the transformed plant cells must be regenerated.

Plant regeneration from cultured protoplasts is described in Evans et al., Handbook of Plant Cell Cultures, Vol. 1: (MacMillan Publishing Co., New York, 1983); and Vasil I.R. (ed.), Cell Culture and Somatic Cell Genetics of Plants, Acad. Press, Orlando, Vol. I, 1984, and Vol. III (1986), which are hereby incorporated by reference.

It is known that practically all plants can be regenerated from cultured cells or tissues, including but not limited to, all major species of sugarcane, sugar beets, cotton, fruit trees, and legumes.

Means for regeneration vary from species to species of plants, but generally a suspension of transformed protoplasts or a petri plate containing transformed explants is first provided. Callus tissue is formed and shoots may be induced from callus and subsequently rooted. Alternatively, embryo formation can be induced in the callus tissue. These embryos germinate as natural embryos to form plants. The culture media will generally contain various amino acids and hormones, such as auxin and cytokinins. It is also advantageous to add glutamic acid and proline to the medium, especially for such species as corn and alfalfa. Efficient regeneration will depend on the medium, on the genotype, and on the history of the culture. If these three variables are controlled, then regeneration is usually reproducible and repeatable.

After the expression cassette is stably incorporated in transgenic plants, it can be transferred to other plants by sexual crossing. Any of a number of standard breeding techniques can be used, depending upon the species to be crossed.

Once transgenic plants of this type are produced, the plants themselves can be cultivated in accordance with conventional procedure with the presence of the gene encoding the fragment of the hypersensitive response elicitor resulting in disease resistance, enhanced plant growth, and/or control of insects on the plant. Alternatively, transgenic seeds or propagules (e.g., cuttings) are recovered from the transgenic plants. The seeds can then be planted in the soil and cultivated using conventional procedures to produce transgenic plants. The transgenic plants are propagated from the planted transgenic seeds under conditions effective to impart disease resistance to plants, to enhance plant growth, and/or to control insects. While not wishing to be bound by theory, such disease resistance, growth enhancement, and/or insect control may be RNA mediated or may result from expression of the polypeptide or protein fragment.

When transgenic plants and plant seeds are used in accordance with the present invention, they additionally can be treated with the same materials as are used to treat the plants and seeds to which a fragment of a hypersensitive response elicitor in accordance with the present invention is applied. These other materials, including a fragment of a hypersensitive response elicitor in accordance with the present invention, can be applied to the transgenic plants and plant seeds by the above-noted procedures, including high or low pressure spraying, injection, coating, and immersion. Similarly, after plants have been propagated from the transgenic plant seeds, the plants may be treated with one or more applications of the fragment of a hypersensitive response elicitor in accordance with the present invention to impart disease resistance, enhance growth, and/or control insects. Such plants may also be treated with conventional plant treatment agents (e.g., insecticides, fertilizers, etc.).

EXAMPLES

Example 1 - Bacterial Strains and Plasmids

Escherichia coli strains used in the following examples include DH5α and BL21(DE3) purchased from Gibco BRL (Grand Island, N.Y.) and Stratagene

(La Jolla, CA), respectively. The pET28(b) vector was purchased from Novagen (Madison, WI). Eco DH5α/2139 contained the complete hrpN gene. The 2139 construct was produced by D. Bauer at Cornell University. The hrpN gene was cleaved from the 2139 plasmid by restriction enzyme digestion with HindIII, then purified from an agarose gel to serve as the DNA template for PCR synthesis of truncated hrpN clones. These clones were subsequently inserted into the $(His)_6$ vector pET28(b) which contained a Kan^r gene for selection of transformants.

Example 2 - DNA Manipulation

Restriction enzymes were obtained from Boehringer Mannheim (Indianapolis, IN) or Gibco BRL. T4 DNA ligase, Calf Intestinal Alkaline Phosphatase (CIAP), and PCR Supermix™ were obtained from Gibco BRL. The QIAprep Spin Miniprep Kit, the Qiagen Plasmid Mini Kit, and the QIAquick PCR Purification Kit were purchased from Qiagen (Hilden, Germany). The PCR primers were synthesized by Lofstrand Labs Limited (Gaithersburg, MD). The oligopeptides were synthesized by Bio-Synthesis, Inc. (Lewisville, TX). All DNA manipulations such as plasmid isolation, restriction enzyme digestion, DNA ligation, and PCR were performed according to standard techniques (Sambrook, et al., Laboratory Manual, Second Edition, Cold Spring Harbor Laboratory Press (1989)) or protocols provided by the manufacturer.

Example 3 - Fragmentation of hrpN Gene

A series of N-terminal and C-terminal truncated *hrpN* genes and internal fragments were generated via PCR (Fig. 1). The full length hrpN gene was used as the DNA template and 3' and 5' primers were designed for each truncated clone (Fig. 2). The 3' primers contained an NdeI enzyme cutting site which contained the start codon ATG (methionine) and the 5' primers contained the stop codon TAA and a HindIII enzyme cutting site for ligation into the pET28(b) vector. PCR was carried out in 0.5 ml tubes in a GeneAmp™ 9700 (Perkin-Elmer, Foster City, CA). 45 µl of Supermix™ (Life Technology, Gaithersburg, MD) were mixed with 20 pmoles of each pair of DNA primers, 10 ng of full length harpin DNA, and deionized

H_2O to a final volume of 50 µl. After heating the mixture at 95°C for 2 min, the PCR was performed for 30 cycles at 94°C for 1 min, 58°C for 1 min and 72°C for 1.5 min. The PCR products were verified on a 6% TBE gel (Novex, San Diego, CA). Amplified DNA was purified with the QIAquick PCR purification kit, digested with Nde I and Hind III at 37°C for 5 hours, extracted once with phenol:chloroform:isoamylalcohol (25:25:1) and precipitated with ethanol. 5 µg of pET28(b) vector DNA were digested with 15 units of Nde I and 20 units of Hind III at 37°C for 3 hours followed with CIAP treatment to reduce the background resulting from incomplete single enzyme digestion. Digested vector DNA was purified with the QIAquick PCR purification kit and directly used for ligation. Ligation was carried out at 14-16°C for 5-12 hours in a 15 µl mixture containing ca. 200 ng of digested pET28(b), 30 ng of targeted PCR fragment, and 1 unit T4 DNA ligase. 5 - 7.5 µl of ligation solution were added to 100 µl of DH5α competent cells in a 15 ml Falcon tube and incubated on ice for 30 min. After a heat shock at 42°C for 45 seconds, 0.9 ml SOC solution or 0.45 ml LB media were added to each tube and incubated at 37°C for 1 hour. 20, 100, and 200 µl of transformed cells were placed onto LB agar with 30 µg/ml of kanamycin and incubated at 37°C overnight. Single colonies were transferred to 3 ml LB-media and incubated overnight at 37°C. Plasmid DNA was prepared from 2 ml of culture with the QIAprep Miniprep kit (QIAGEN, Hilden, Germany). The DNA from the transformed cells was analyzed by restriction enzyme digestion or partial sequencing to verify the success of the transformations. Plasmids with the desired DNA sequence were transferred into the BL21 strain using the standard chemical transformation method as indicated above. A clone containing the full length harpin protein in the pET28(b) vector was generated as a positive control, and a clone with only the pET28(b) vector was generated as a negative control.

Example 4 - Expression of Hypersensitive Response Elicitor Truncated Proteins

Escherichia coli BL21(DE3) strains containing the hrpN clones were grown in Luria broth medium (5 g/L Difco Yeast extract, 10 g/L Difco Tryptone, 5 g/L NaCl, and 1 mM NaOH) containing 30 µg/ml of kanamycin at 37°C overnight. The bacteria were then inoculated into 100 volumes of the same medium and grown at

37°C to an OD_{620} of 0.6-0.8. The bacteria were then inoculated into 250 volumes of the same medium and grown at 37°C to an OD_{620} of ca. 0.3 or 0.6-0.8. One milli molar IPTG was then added and the cultures grown at 19°C overnight (ca. 18 hours). Not all of the clones were successfully expressed using this strategy. Several of the clones had to be grown in Terrific broth (12 g/L Bacto Tryptone, 24 g/L Bacto yeast, 0.4% glycerol, 0.17 M KH_2PO_4, and 0.72 K_2HPO_4), and/or grown at 37°C after IPTG induction, and/or harvested earlier than overnight (Table 1).

Table 1: Expression of hypersensitive response elicitor truncated proteins

Fragment	amino acids (SEQ. ID. No. 23)	Growth medium	Induction O.D.	Expression temp.	Harvest time
1 (+ control)	1-403	LB	ca. 0.3 or 0.6-0.8	19°C or 25°C	16-18 hr
2 (+ control)	-	LB and TB	ca. 0.3 or 0.6-0.8	19 C and 37 C	16-18 hr
3	105-403	LB	0.6-0.8	19°C	16-18 hr
4	169-403	TB	ca. 0.3	19°C	16-18 hr
5	210-403	LB or M9ZB	0.6-0.8	19°C	16-18 hr
6	257-403	LB or M9ZB	0.6-0.8	19°C	16-18 hr
7	343-403	LB	ca. 0.3	19°C	5 hr
8	1-75	TB	ca. 0.3	37°C	16-18 hr
9	1-104	TB	ca. 0.3	37°C	16-18 hr
10	1-168	TB	ca. 0.3	37°C	16-18 hr
11	1-266	LB	ca. 0.3	37°C	4 hr
12	1-342	LB	0.6-0.8	19°C	16-18 hr
13	76-209	LB	ca. 0.3	37°C	5 hr
14	76-168	TB or LB	ca. 0.3	37°C	3 hr or 16-18 hr
15	105-209	M9ZB	ca. 0.3	37°C	3 hr
16	169-209	no expression			
17	105-168	LB	ca. 0.3	37°C	3-5 hr
18	99-209	LB	ca. 0.3	37°C	3 hr
19	137-204	LB	ca. 0.3	37°C	3 hr
20	137-180	LB	ca. 0.3	37°C	16-18 hr.
21	105-180	LB	ca. 0.3	37°C	3 hr
22	150-209	no expression			
23	150-180	no expression			

Example 5 - Small Scale Purification of Hypersensitive Response Elicitor Truncated Proteins (Verification of Expression)

A 50 ml culture of a hrpN clone was grown as above to induce expression of the truncated protein. Upon harvesting of the culture, 1.5 ml of the cell

suspension were centrifuged at 14,000 rpm for 5 minutes, re-suspended in urea lysis buffer (8 M urea, 0.1 M Na_2HPO_4, and 0.01 M Tris -- pH 8.0), incubated at room temperature for 10 minutes, then centrifuged again at 14,000 rpm for 10 minutes, and the supernatant saved. A 50 µl aliquot of a 50% slurry of an equilibrated $(His)_6$-binding nickel agarose resin was added to the supernatant and mixed at 4°C for one hour. The nickel agarose was then washed three times with urea washing buffer (8 M urea, 0.1 M Na_2HPO_4, and 0.01 M Tris -- pH 6.3), centrifuging at 5,000 rpm for five minutes between washings. The protein was eluted from the resin with 50 µl of urea elution buffer (8 M urea, 0.1 M Na_2HPO_4, 0.01 M Tris, and 0.1 M EDTA -- pH 6.3). The eluate was run on a 4-20%, a 16%, or a 10-20% Tris-Glycine pre-cast gel depending upon the size of the truncated protein to verify the expression.

Example 6 - Induction of HR in Tobacco

A 1.5 ml aliquot from the 50 ml cultures grown for small scale purification of the truncated proteins was centrifuged at 14,000 rpm for four minutes and re-suspended in an equal volume of 5 mM potassium phosphate buffer, pH 6.8. The cell suspension was sonicated for ca. 30 seconds then diluted 1:2 and 1:10 with phosphate buffer. Both dilutions plus the neat cell lysate were infiltrated into the fourth to ninth leaves of 10-15 leaf tobacco plants by making a hole in single leaf panes and infiltrating the bacterial lysate into the intercellular leaf space using a syringe without a needle. The HR response was recorded 24-48 hr post infiltration. Tobacco (*Nicotiana tabacum* v. Xanthi) seedlings were grown in an environmental chamber at 20-25°C with a photoperiod of 12-h light /12-h dark and ca. 40% RH. Cell lysate was used for the initial HR assays (in order to screen the truncated proteins for HR activity) as the small scale urea purification yielded very little protein which was denatured due to the purification process.

Example 7 - Large Scale Native Purification of Hypersensitive Response Elicitor Truncated Proteins for Comprehensive Biological Activity Assays

Six 500 ml cultures of a hrpN clone were grown as described earlier to induce expression of the truncated protein. Upon harvesting of the culture, the cells were centrifuged at 7,000 rpm for 5 minutes, re-suspended in imidazole lysis buffer (5

mM imidazole, 0.5 M NaCl, 20 mM Tris) plus Triton X-100 at 0.05% and lysozyme at 0.1 mg/ml, incubated at 30°C for 15 minutes, sonicated for two minutes, centrifuged again at 15,000 rpm for 20 minutes, and the supernatant was saved. A 4 ml aliquot of a 50% slurry of an equilibrated $(His)_6$-binding nickel agarose resin was added to the supernatant and mixed at 4°C for ca. four hours. The nickel agarose was then washed three times with imidazole washing buffer (20 mM imidazole, 0.5 M NaCl, and 20 mM Tris), centrifuging at 5,000 rpm for five minutes between washings, then placed in a disposable chromatography column. The column was centrifuged at 1100 rpm for one minute to remove any residual wash buffer and then the protein was eluted from the resin with 4 ml of imidazole elution buffer (1 M imidazole, 0.5 M NaCl, and 20 mM Tris) by incubating the column with the elution buffer for ten minutes at room temperature and then centrifuging the column at 1100 rpm for one minute. The eluate was run on a 4-20%, a 16%, or a 10-20% Tris-Glycine pre-cast gel depending upon the size of the truncated protein to verify the expression. The concentration of the proteins was determined by comparison of the protein bands with a standard protein in the Mark 12 molecular weight marker.

Example 8 - Large Scale Urea Purification of Hypersensitive Response Elicitor Truncated Proteins For Comprehensive Biological Activity Assay

The procedure was the same as the large scale native purification except that urea lysis buffer, washing buffer, and elution buffer were used, and the cells were not sonicated as in the native purification. After purification, the protein was renatured by dialyzing against lower and lower concentrations of urea over an eight hour period, then dialyzing overnight against 10 mM Tris/20 mM NaCl. The renaturing process caused the N-terminal proteins to precipitate. The precipitated 1-168 protein was solubilized by the addition of 100 mM Tris-HCl at pH 10.4 then heating the protein at 30°C for ca. one hour. The concentration of the protein was determined by comparison of the protein bands with a standard protein in the Mark 12 molecular weight marker. The 1-75 and 1-104 protein fragments were not successfully solubilized using this strategy so they were sonicated in 100 mM Tris-HCl at pH 10.4 to solubilize as much of the protein as possible and expose the active sites of the protein for the biological activity assays.

Example 9 – Induction of Growth Enhancement (GE)

Sixty tomato (*Lycopersicon spp.* cv. Marglobe) seeds were soaked overnight in 10 and 20 μg/ml of the truncated protein diluted with 5mM potassium phosphate buffer, pH 6.8. The next morning, the sixty seeds were sewn in three pots and 12-15 days later and again 18-20 days later the heights of the 10 tallest tomato plants per pot were measured and compared with the heights of the control plants treated only with phosphate buffer. Analyses were done on the heights to determine if there was a significant difference in the height of the plants treated with the truncated proteins compared with the buffer control, and thereby determine whether the proteins induced growth enhancement.

Example 10 – Induction of Systemic Acquired Resistance (SAR)

Three tobacco (*Nicotiana tabacum* cv. Xanthi) plants with 8-12 leaves (ca. 75 day old plants) were used in the assay. One leaf of the tobacco plants was covered up and the rest of the leaves were sprayed with ca. 50 ml of a 20 μg/ml solution of the truncated proteins diluted with 5mM potassium phosphate buffer. Five to seven days later two leaves (the unsprayed leaf and the sprayed leaf opposite and just above the unsprayed leaf) were inoculated with 20 μl of a 1.8 μg/ml solution of TMV along with a pinch of diatomaceous earth by rubbing the mixture along the top surface of the leaves. The TMV entered the plants through tiny lesions made by the diatomaceous earth. Ca. 3-4 days post TMV inolucation, the number of TMV lesions was counted on both leaves compared with the number of lesions on the negative control buffer treated leaves. Analyses were done to determine the efficacy of reducing the number of TMV lesions by the protein fragments compared to the buffer control. Percentage of efficacy was calculated as: Reduction in TMV lesions (% efficacy) = 100 x (1 – mean # of lesions on treated leaves/mean # of lesions on buffer control leaves).

Example 11 - Expression of Hypersensitive Response Elicitor Truncated Proteins

The small scale expression and purification of the fragment proteins was done to screen for expression and HR activity (Table 2).

Table 2

Expression and HR activity of hypersensitive response elicitor truncated proteins (small scale screening)

Fragment #	Amino Acids (SEQ. ID. No. 23)	Expression	HR activity
1(+control)	1-403	+	+
2(- control)	-	background protein only	-
3	105-403	+	+
4	169-403	+	-
5	210-403	+	-
6	267-403	+	-
7	343-403	+/-	-
8	1-75	+	-
9	1-104	+	+/-
10	1-168	+	+
11	1-266	+	+
12	1-342	+	+
13	76-209	+	+
14	76-168	+	-
15	105-209	+	+
16	169-209	-	-
17	105-168	+	-
18	99-209	+	+
19	137-204	+	+
20	137-180	+	+
21	105-180	+	+
22	150-209	-	-
23	150-180	-	-

All of the cloned fragment proteins were expressed at varying levels except for three small fragments (amino acids 169-209, 150-209, and 150-180). Fragments 210-403 and 267-403 were expressed very well, yielding a high concentration of protein from a small scale purification, resulting in a substantial protein band on SDS gel electrophoresis. Other fragments (such as a.a. 1-168 and 1-104) produced much less protein, resulting in faint protein bands upon electrophoresis. It was difficult to determine whether fragment 343-403, the smallest C-terminal protein, was expressed, as there were several background proteins apparent on the gel, in addition to the suspected 343-403 protein. The positive and negative control proteins, consisting of

the full length hypersensitive response elicitor protein and only background proteins, respectively, were tested for expression and HR activity as well.

The large scale expression and purification of the fragment proteins was done to determine the level of expression and titer of the HR activity (Table 3).

Table 3

Expression level and HR titer of hypersensitive response elicitor truncated proteins (large sale purification)

Fragment #	Amino acids (SEQ. ID. No. 23)	Expression	HR titer
1(+ control)	1-403	3.7 mg/ml	5-7 µg/ml
2 (- control)	-	-	1:2 dilution
4	169-403	2 mg/ml	-
5	210-403	5 mg/ml	-
6	267-403	4 mg/ml	-
7	343-402	200µg/ml	-
8	1-75	50µg/ml	-
9	1-104	50µg/ml	3 µg/ml (1:16 dilution)
10	1-168	1 mg/ml	1 µg/ml
13	76-209	2.5 mg/ml	5 µg/ml
14	76-168	2 mg/ml	-
15	105-209	5 mg/ml	5-10µg/ml
17	105-168	250µg/ml	-
19	137-204	3.6 mg/ml	3.5 µg/ml
20	137-180	250 µg/ml	16 µg/ml

The truncated proteins deemed to be the most important in characterizing the hypersensitive response elicitor were chosen for large scale expression. The positive control (full length hypersensitive response elicitor) was expressed at a relatively high level at 3.7 mg/ml. All of the C-terminal proteins were expressed at relatively high levels from 2-5 mg/ml, except for fragment 343-403 as discussed earlier. The N-terminal fragments were expressed very well also; however, during the purification process, the protein precipitated and very little was resolubilized. The concentrations in Table 3 reflect only the solubilized protein. The internal fragments were expressed in the range of 2-3.6 mg/ml. It was extremely difficult to determine the concentration of fragment 105-168 (it was suspected that the concentration was much higher than indicated), as the protein bands on the SDS gel were large, but poorly stained. The

negative control contained several background proteins as expected, but no obviously induced dominant protein.

Example 12 - Induction of HR in Tobacco

The full length positive control protein elicited HR down to only 5-7μg/ml. The negative control (pET 28) imidazole purified "protein" - which contained only background proteins - elicited an HR response down to the 1:2 dilution, which lowered the sensitivity of the assay as the 1:1 and 1:2 dilutions could not be used. This false HR was likely due to an affinity of the imidazole used in the purification process to bind to one or several of the background proteins, thereby not completely dialyzing out. Imidazole at a concentration of ca. 60 mM did elicit a false HR response.

One definitive domain encompassing a small internal region of the protein from a.a. 137-180 (SEQ. ID. No. 23), a mere 44 a.a, is identified as the smallest HR domain. The other potential HR domain is thought to be located in the N-terminus of the protein from a.a. 1-104 (possibly a.a. 1-75) (SEQ. ID. No. 23). It was difficult to confirm or narrow down the N-terminus HR domain due to the difficulties encountered in purifying these fragment proteins. The N-terminus fragment proteins had to be purified with urea as no protein was recovered when the native purification process was used. Consequently, these proteins precipitated during the renaturing process and were difficult or nearly impossible to get back into solution, thereby making it hard to run the proteins through the HR assay, as only soluble protein is able to elicit HR. Difficulty narrowing the N-terminus HR domain was only compounded by the fact that the negative control elicited false HR at the low dilution levels thereby reducing the sensitivity of the assay.

Surprisingly, when the internal HR domain was cleaved between a.a. 168 and 169 (fragments 76-168 and 105-168) (SEQ. ID. No. 23) the fragment lost its HR activity. This suggests that the HR activity of fragment 1-168 (SEQ. ID. No. 23) should not be attributed to the internal HR domain, but rather to some other domain, leading to the assumption that there was likely a second HR domain to be found in the N-terminal region of the protein. However, as discussed earlier it was difficult to confirm this assumption.

The hypersensitive response elicitor C-terminus (a.a. 210-403 (SEQ. ID. No. 23)) did not contain an HR domain. It did not elicit HR at a detectable level using the current HR assay. Even the large C-terminal fragment from a.a. 169-403 (SEQ. ID. No. 23) did not elicit HR even though it contained part of the internal HR domain. As stated above, cleaving the protein between amino acids 168 and 169 (SEQ. ID. No. 23) causes a loss of HR activity.

Because some of the small cloned proteins with 61 a.a. or less were not expressed, several oligopeptides were synthesized with 30 a.a. to narrow down the functional region of the internal HR domain. The oligopeptides were synthesized within the range of a.a. 121-179 (SEQ. ID. No. 23). However, these oligos did not elicit HR. It was not expected that there would be an HR from oligos 137-166, 121-150, and 137-156 (SEQ. ID. No. 23) as these fragments did not contain the imperative amino acids 168 and 169 (SEQ. ID. No. 23). It was expected that the oligo 150-179 (SEQ. ID. No. 23) would elicit an HR. It is possible that 30 a.a. is too small for the protein to elicit any activity due to a lack of folding and, therefore, a lack of binding or that during the synthesis of the peptides important amino acids were missed (either in the process, or simply by the choice of which 30 amino acids to synthesize) and, therefore, the fragments would not be able to elicit HR.

<u>Example 13</u> – Induction of Plant Growth Enhancement (PGE)

The C-terminal fragments enhanced the growth of tomato by 9% to 21%. The N-terminal fragments enhanced the growth of tomato by 4% to 13%. The internal fragments enhanced growth by 9% to 20%. The 76-209 fragment enhanced growth by 18% at a concentration of 60 μg/ml, but not at the typical 20 μg/ml. This was attributed to the inaccuracy of the quantification process (Table 4).

Table 4

Fragment #	Amino acids	PGE ht>buffer @ 10 μg/ml	PGE ht>buffer @ 20 μg/ml
1 (+ control)	1-403	12%	11%
2 (- control)	-	-3%	-2%
4	169-403	9%	12%
5	210-403	13%	14% 16% @ 40μg/ml
6	267-403	21%	21% 23% @ 40μg/ml
7	343-403	7%	7%
9	1-104	4%	8%
10	1-168	13%	5%
13	76-209	7%	4% 18% @ 60μg/ml
14	76-168	18%	20%
15	105-209	14%	19%
17	105-168	19%	16%
19	137-204	11%	13%
20	137-180	--	9%

*A height greater than 10% above the buffer control was necessary to pass the PGE assay.

The oligopeptides enhanced growth from 7.4% to 17.3% (Table 5).

Table 5

Fragment	Amino acids	Expression	HR titer	TMV efficacy	PGE ht>buffer
oligo	150-179	NA	-	72.9%	10.1%
oligo	137-166	NA	-	61.2%	12.0%
oligo	121-150	NA	-	60.0%	17.3%
oligo	137-156	NA	-	-87.7%	7.4%

The data suggests that there is more than one PGE domain, although the C-terminal and internal domains appear to be dominant over the N-terminal domain, as the N-terminal fragments enhanced growth the least amount.

Example 14 – Induction of Systemic Acquired Resistance (SAR)

All of the hypersensitive response elicitor fragments tested to date appear to have 60% efficacy or greater, except for the oligopeptide 137-156 (Tables 5 and 6).

Table 6

Fragment #	Amino acids	Efficacy of TMV control
1 (+ control)	1-403	84% & 72%
2 (- control)	-	40% & 31%
4	169-403	64% & 79%
5	210-403	77% and 78%
6	267-403	70% and 72%
9	1-104	82%
10	1-168	69%
13	76-209	44% and 84%
14	76-168	83% & 87%
15	105-209	57% and 67%
17	105-168	89%
19	137-204	89% & 77%
20	137-180	64% & 58%

These data suggest that there are multiple SAR domains within the protein.

Example 15 – Relationship Between HR, PGE, and SAR

It is clear that the hypersensitive response activity is separable from the plant growth enhancement activity. The C-terminal fragments clearly enhance the growth of tomato by ca. 20% at a concentration of only 20 μg/ml, but these same fragments were not able to elicit HR in tobacco, even at higher concentrations than 200 μg/ml. The SAR activity also appears to be separable from the HR activity. This finding is highly significant for future work on transgenic applications of the hypersensitive response elicitor technology. The fragments that induce PGE and/or SAR but do not elicit HR will be imperative for this technology, as constitutive expression of even low levels of an HR elicitor might kill a plant.

Example 16 - Non-HR Eliciting Fragments Derived from the Hypersensitive Response Elicitor from *Pseudomonas syringae* pv. *syringae* Induce Resistance in Tobacco to TMV and Promote the Growth of Tomato

To test whether non-HR eliciting fragments derived from HrpZ, the hypersensitive response elicitor from *Pseudomonas syringae* pv. *syringae*, is able to induce disease resistance, several fragment constructs were made and the expressed

fragment proteins were tested for HR elicitation and disease resistance induction in tobacco and growth promotion in tomato.

The following segments of *hrpZ*, the gene encoding the hypersensitive response elicitor from *Pseudomonas syringae* pv. *syringae*, were amplified by PCR using Pfu Turbo (Stratagene): Regions coding for amino acids 152-190, aa 152-294, aa 190-294, aa 301-341, and full length HrpZ (aa 1-341). The DNA fragments were cloned into pCAL-n (Stratagene) to create C-terminal fusion proteins to the calmodulin-binding peptide. pCAL-n was chosen, because the fusion protein could be easily and gently purified on calmodulin resin. The DNA was transformed into *E. coli* DH5α, and the correct clones were identified. The clones were then transferred to *E. coli* BLR DE3 for protein expression. The bacteria were grown in Terrific Broth to an OD_{620} of 0.8-1.0. Protein expression was then induced with IPTG and the bacteria were incubated for an additional 3 h. All of the HrpZ fragments were able to be expressed this way.

Amino acid fragments 152-294 and 190-294 were chosen for further analysis and characterization. It was expected that the fragment 152-294 contained a domain that elicited the HR, while fragment 190-294 contained no domain that elicited the HR. The cultures were spun down, and the bacteria resuspended in 40 ml of 10 mM Tris pH 8.0. Twenty µl of antifoam and 40 µl of 200 mM PMSF were added, and the bacteria was sonicated to break open the cells. The bacterial debris was removed by centrifugation, and the supernatant was placed in a boiling water bath for 10 min. The precipitate was removed by centrifugation and the supernatant, a crude protein preparation, was retained for tests.

Fifteen µl of each supernatant was run on a gel and stained to determine if the protein was present. It was estimated that about five times as much of the 152-294 fragment was present as the 190-294 fragment. Several dilutions of each preparation were infiltrated into tobacco leaves on two plants for HR tests (Table 7). As shown in Table 7, the 152-294 fragment elicited an HR, but the 190-294 fragment did not.

Table 7

HR test results of HrpZ fragments

HrpZ Fragment	Dilution of Fragment Preparation[a]			
	1:2	1:5	1:25	1:125
152-294	+,+[b]	+,+	+,+	–,–
190-294	–,–	–,–	–,–	–,–

[a] The preparations were diluted with MilliQ water.
[b] The results are indicated for each of two plants. +, HR; –, no HR.

The fragment preparations were then tested for inducing resistance to TMV and for growth enhancement. Due to the difference in concentration of the HrpZ fragments, the 152-294 preparation was diluted 40-fold and the 190-294 preparation was diluted 8-fold. The results showed that the 190-294 aa fragment reduced the number of TMV lesions by 85% in comparison to buffer controls (Table 8). In contrast, the 152-294 aa fragment reduced the number of TMV lesions by only 55%. As also shown in Table 8, plants treated with the 152-294 aa fragment grew 4.64% more than buffer treated plants, while plants treated with the 190-294 aa fragment grew 2.62% more than the buffer treated plants.

Table 8

HR test, TMV, and PGE test results

HrpZ Fragment	HR elicitation[a]	TMV (% efficacy)[b]	PGE(% > buffer ht)[c]
152-294	+	54.64	4.64
190-294	–	85.25	2.62

[a] +, elicits HR in tobacco leaves; –, no HR in tobacco leaves.
[b] % reduction in TMV lesions in unsprayed leaf of tobacco.
[c] % greater height than buffer sprayed plants.

The results of these tests show that amino acids 152-190 appear to be involved in HR elicitation, because their removal eliminated the ability to elicit the HR. Both fragment preparations achieved disease control and growth enhancement. Thus, the ability to elicit the HR is not the determining factor for reduction in TMV infection and growth enhancement.

Example 17 - Use of 13 Amino Acid Peptide Derived from *Phytophthora megasperma* Stimulates Tomato Seedling Growth

Parsley leaves develop a typical resistance reaction against the soybean pathogen *Phytophthora megasperma* comprising hypersensitive cell death, defense related gene activation, and phytoalexin formulation. Several years ago, a 42 kDa glycoprotein elicitor was purified from the fungal culture filtrate of *Phytophthora megasperma* (Parker et al., "An Extracellular Glycoprotein from *Phytophthora megasperma* f.sp. glycinea Elicits Phytoalexin Synthesis in Cultured Parsley Cells and Protoplasts," Mol. Plant Microbe Interact. 4:19-27 (1991), which is hereby incorporated by reference). Then, an oligopeptide of 13 amino acid was identified within the 42 kDa glycoprotein. The 13 amino acids peptide appeared to have similar biological activity as that of the full length glycoprotein (42 kDa). It is sufficient to elicit a complex defense response in parsley cells including H+/Ca2+ influxes, K+/Cl- effluxes, active oxygen production, SAR gene induction, and phytoalexin compound accumulation (Nurnberger et al., "High Affinity Binding of a Fungal Oligopeptide Elicitor to Parsley Plasma Membranes Triggers Multiple Defense Response," Cell 78:449-460 (1994), which is hereby incorporated by reference).

To test if the 13 amino acid peptide derived from the 42 kDa protein also enhanced plant growth, 20 mg of the oligopeptide was synthesized from Biosynthesis Corp. The synthesized sequence of the peptide is NH2-Val-Trp-Asn-Gln-Pro-Val-Arg-Gly-Phe-Lys-Val-Tyr-Glu-COOH (SEQ. ID. No. 39). The synthesized peptide was resuspended in 10 ml of 5 mM potassium phosphate buffer and, then, diluted to 1 and 100 ng/ml with the same buffer. About 100 tomato seeds (variety, Marglobe) were submerged in 20 ml of peptide solution overnight. The soaked seeds were planted in an 8 inch pot with artificial soil. Seeds soaked in the buffer without the peptide were used as a control. After seedlings emerged and the first two true leaves fully expanded, the height of the tomato seedlings was recorded. The peptide was not able to elicit the HR in tobacco and other tested plants. However, it had a profound effect on plant growth promotion. Table 9 shows that tomato seedlings treated with the peptide increased 12.6 % in height, indicating that the fungal peptide derived from the 42 kDa glycroprotein can promote tomato seedling growth. Extended studies showed that the peptide also had similar growth

effect in other crops including tobacco. Similar growth promotion effects were achieved by plants sprayed with the peptide solution.

Table 9

Treatment	Height of seedlings (cm)					Average (cm)	% Change
Buffer	6.0	6.0	6.0	5.5	5.5	5.55	-
	5.5	5.5	5.0	5.0	5.5		
Peptide Solution (100ng/ml)	6.5	6.0	6.5	6.5	6.5	6.25	12.6
	6.0	6.0	6.0	6.0	6.5		

Although the invention has been described in detail for the purpose of illustration, it is understood that such detail is solely for that purpose, and variations can be made therein by those skilled in the art without departing from the spirit and scope of the invention which is defined by the following claims.